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Fatty acid modification of cells in culture by overexpression of exogenous desaturases

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Fatty acid modification of cells in culture by overexpression of exogenous desaturases

by

Travis Jay Knight

A dissertation submitted to the graduate faculty

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This is to certify that the Doctoral dissertation of

Travis Jay Knight

Has met the dissertation requirements of Iowa State University

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Major Professor

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For the Major Program

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For the Graduate College

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GENERAL INTRODUCTION

Dissertation Organization

This dissertation is presented in an alternative format. Following the General Introduction, a Literature Review on topics related to the dissertation research is presented. A manuscript prepared for publication is complete in itself with an abstract, introduction, material and methods, results, discussion, and references cited. A General Conclusion follows the paper and presents in brief the major finding and conclusion of the dissertation research. References cited in the General Introduction and Literature Review are listed following the General Conclusion.

Background and Significance

Fatty acid desaturation occurs in plants, animals, insects, microbes, and fungi. The need for an organism to modify preformed or *de novo* synthesized lipids is multifactorial. The correct ratio and amount of the various fatty acids are important in maintaining metabolic functions in all organisms related to reproduction, inflammation, and immunology from both a metabolite and from a structural membrane integrity viewpoint. A primary function of fatty acid desaturases is to maintain proper membrane fluidity as other nutritional, environmental, or physiological changes occur. The ratio of 18:0 to 18:1(n-9) is an example of a factor influencing cell membrane fluidity. Aging, obesity, cancer, diabetes, and heart disease all have been associated with changes in membrane fatty acid composition (Spector, 1985).

There are three types of fatty acid desaturases—acyl-Coenzyme A (CoA) desaturases, acyl-carrier protein (ACP) desaturases, and acyl-lipid desaturases (Murata and Wada, 1995). Acyl-ACP desaturases are found in the stroma of plant tissue (McKeon and Stumpf, 1982). Acyl-CoA desaturases are found in the endoplasmic reticulum membrane of animal cells, yeast, and other fungal cells (Holloway, 1983). Acyl-lipid desaturases are found bound to the endoplasmic reticulum and the chloroplast membranes of plant cells (Schmidt and Heinz, 1993) and the thylakoid membranes of cyanobacterial cells (Wada et al., 1993). This review will focus on modes of regulation of acyl-CoA desaturases and mechanisms of acyl-ACP desaturases of plants and the acyl-CoA desaturases of animals, yeast, and other fungi. The underlying thought behind this review and research project is on modifying the fatty acid composition of organisms for improving food quality or for production of lipids with increased industrial value.

Type and amount of dietary fat have been linked to several disease states, including atherosclerosis (Phillipson et al., 1985), insulin-resistant diabetes (Storlien et al., 1987), and certain types of cancers (Cave, 1991; Welsch, 1992). Fatty acids can exert their influence on disease in the long-term by changing the fatty acid composition of membranes and altering the physical character of the membrane and the proteins that are associated with the membrane (Spector and York, 1985). More recently, immediate effects of dietary fatty acids on nuclear events that govern gene transcription have been examined (Jump et al., 1995). Clarke and Jump (1996a) authored a review on the effects of polyunsaturated fatty acid (PUFA) regulation of hepatic gene transcription, with particular emphasis on 20:4(n-6). In that review, they focused on proof that the newly cloned fatty acid-activated nuclear factor,

named peroxisome proliferator-activated receptor (PPAR), acts independently of a yet-to-be identified PUFA response factor in controlling lipid-responsive genes.

As the effects of specific fatty acids on disease conditions are determined, the need to modify fatty acid composition of the human diet will increase. Many examples of such modifications are already evident in plant-derived oils such as high-18:1(n-9) soybean oil and low-22:1(n-9) (erucic acid) canola oil. Animal products, the major source of both fat and protein in typical human diets, supply 55% of dietary fat, 64% of dietary saturated fatty acids, and 70% of dietary protein (National Livestock and Meat Board, A Dietary Pattern and Intake Report, 1994). The major sources of animal fat in the American diet are beef, pork, and milk fat, which all contain around 50% saturated fatty acids. Changing fatty acid composition of muscle tissue in monogastric animals is fairly easy compared with that of ruminants because dietary fatty acids are absorbed and used in membrane synthesis and for energy storage in the same form that they exist in the diet. In ruminants, however, dietary unsaturated fatty acids are saturated in the highly reductive rumen before absorption (Ashes et al., 1997). Thus, ruminant fatty acid desaturase systems control milk and meat fatty acid composition.

Gibson's 1991 review summarizes the potential genetic means for changing fatty acid composition in milk fat. There are modest differences of milk fatty acid composition between and within breeds, indicating that traditional breeding practices could use selection criteria to develop genetic strains to produce milk with specific fatty acid composition. Another approach would be to manipulate the genes responsible for fatty acid synthesis. Despite the probable nature of success in significantly modifying the fatty acids in milk via traditional or modern methods, the costs associated with such efforts would not be regained

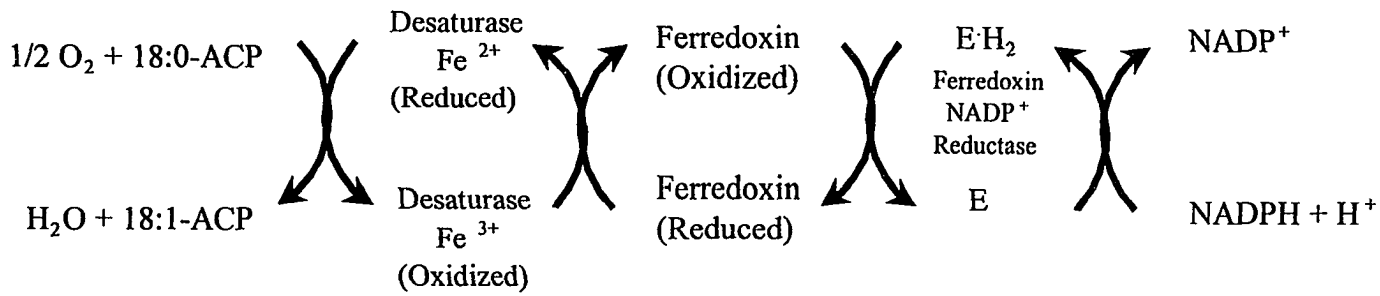
by producers or processors. Therefore, the likelihood of designing specific strains of cows for specific niche markets is unlikely in the near future.

Modifying plants to make fatty acids for special industrial or nutritional applications has been attempted with limited success through traditional plant breeding efforts. More recently, transgenic approaches, as described initially by Somerville and Browse (1991), have been employed to shuttle desirable characteristics of oil production from one plant to another plant that is easier to grow or that produces more triacylglycerol. Cahoon et al. (1997a) have redesigned acyl-carrier protein (ACP) desaturases to learn about the chain-length and positional specificity of this class of soluble desaturases in plants. In one experiment, Δ^6-palmitoyl-ACP desaturase was mutated by replacing key amino acids from Δ^9-stearoyl ACP-desaturase sequence, creating a new series of enzymes capable of desaturating substrates with different number of carbon atoms or at different positions in the acyl chain.

In comparing the soluble stearoyl-ACP desaturase (SACPD) systems in plants and the membrane bound steroyl-CoA desaturase (SCD) system in animals, there are similarities and striking differences (Figure 1). Similarities include insertion of a double bond at the Δ^9 position of 18:0 to form 18:1(n-9) and the requirement of O_2 and an electron transport system to keep the oxidation/reduction of the terminal desaturase (the desaturase enzyme excluding accessory proteins and enzymes) operational. While plant systems rely on ferredoxin and NADPH-ferredoxin-oxidoreductase for their electron transport system, mammals rely on cytochrome b_5 and NADH-cytochrome b_5 reductase. The favored substrates, acyl-CoA in animals and acyl-ACP in plants, are both soluble substrates. Both plant and mammalian Δ^9-desaturases have similar K_m and V_{max} values for stearoyl-CoA, but the kinetics of the plant

Comparison of Desaturase Optimal Cofactors

Plant Systems



Mammalian Systems

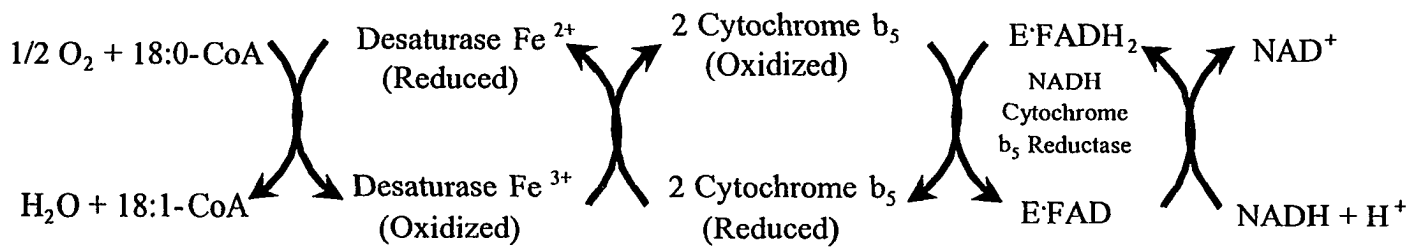


Figure 1. Cofactor requirements of plant and mammalian fatty acid desaturases.

enzyme indicate that stearoyl-CoA would have to be at or above the critical micellar concentration for significant amounts of 18:1(n-9) synthesis to occur (Enoch et al., 1976). But, because of the low concentration of stearoyl-CoA in plant tissue, the plant enzyme acts almost entirely on stearoyl-ACP.

The objectives of the present study were as follows: (1) prepare mammalian expression constructs by using both cDNAs for SCD and SACPD, (2) determine if both SCD and SACPD are transcribed and translated in mammalian cells in culture, and (3) determine if expression of the exogenous desaturases causes changes in fatty acid composition of the cells. The results of this study will provide further insight about expressing plant cDNAs in mammalian systems and, in this specific case, if proteins native to plants can utilize animal cofactors and substrates to function. Modifying fatty acid composition of plants has already been accomplished by utilizing the tools of molecular biology. This research will provide foundational information toward determining the feasibility of using genetic engineering to modify the fatty acid composition of food animals.

LITERATURE REVIEW

Reaction Mechanism

Enzymology

The enzymology of fatty acid desaturases of both plant and animal systems was initiated in the 1960s. When the plant and animal desaturases are compared, they have similar K_m and V_{max} for stearoyl-CoA, but again it was determined that, for the plant enzyme to work appreciably well on stearoyl-CoA, the substrate concentration has to be above the critical micellar concentration for stearoyl-CoA (McKeon and Stumpf 1982). Shanklin and Somerville (1991) proposed that, despite the known difference in primary sequences of plant and mammalian fatty acid desaturase enzymes, the chemical mechanisms were probably similar. The membrane-bound animal enzymes proved to be much more difficult to work with than the soluble plant acyl-ACP desaturases, and much of the reaction mechanism data that has been collected has been from soluble, plant-derived enzymes.

Animal Systems

Jones et al. (1969) described the requirement of lipids by the microsomal SCD system from hen liver. Microsomal preparations when extracted with aqueous acetone lost enzyme activity. But, when the extracted preparations were mixed with phospholipids, triacylglycerols, and fatty acids, the enzyme regained activity. This experimentation also demonstrated a requirement for oxygen and reduced pyridine nucleotide. In 1974, Strittmatter et al. purified rat liver microsomal SCD to homogeneity and determined that it contained one molecule of non-heme iron, which is important in the mechanism as it reacts

with oxygen and reduced cytochyrome b_5. These studies reiterated the importance of reduced cytochrome b_5 and phospholipids. Based on an estimated turnover number of about 21 reactions per enzyme molecule per minute and the activity in induced rat liver microsomes, Strittmatter estimated that 0.7-0.8% of the hepatic microsomal protein was SCD.

Gurr and Robinson (1970) partially purified the liver microsomal SCD from hen liver. A soluble form of the desaturase was prepared by extracting it from freeze-dried microsomes with high ionic strength phosphate buffer or non-ionic detergents. By removing some of the microsomal phospholipids with gentle acetone extractions, relative desaturation of the preparation increased, but, when more than about 75% of the phospholipids were extracted, relative activity decreased. However, reconstitution by the addition of micellar phospholipid could not restore activity.

A method of purification of SCD from rat liver is provided in a 1978 article by Strittmatter and Enoch. The method employs induction of the enzyme by fasting and refeeding rats a high-carbohydrate diet followed by enzyme solubilization with a detergent solution of Triton X-100-calcium deoxycholate. By using this procedure, the authors were able to increase the specific activity by greater that 150-fold, which proved useful in isolating enzyme for future kinetic studies.

Okayasu et al. (1981) partially purified and characterized the Δ^6-desaturase from rat liver microsomes. Triton X-100 solubilization, followed by several rounds of column chromatography and ultimately cytochrome b_5-Sepharose affinity chromatography, resulted in a homogeneous preparation with a single polypeptide of 66 kDa. In a reconstituted system, it was determined that, besides the terminal desaturase, NADH, O_2, linoleoyl-CoA,

lipid or detergent, NADH-cytochrome b_5 reductase, and cytochrome b_5 were required for enzyme activity. Interestingly, NADPH and cytochrome P_{450} reductase could replace NADH and cytochrome b_5 reductase and the reaction would proceed at about 60% of optimal activity. Under ideal conditions, the K_m was 45 μM and the V_{max} was 83 nmol/min•mg protein.

Enoch et al. (1976) did a thorough investigation concerning the mechanism of rat liver SCD by using various substrates and *in vitro* conditions to better understand the enzyme. By combining the three purified proteins required for stearoyl-CoA desaturation—NADH-cytochrome b_5 reductase, cytochrome b_5, and the terminal desaturase—into egg lecithin vesicles, a functional electron transport system with active SCD-activity was available. It is assumed that, because hydrophilic chelating agents could strip the iron from the desaturase, that the active site of the enzyme was oriented on the surface of the vesicle and outward into the aqueous environment. Thus, the cytochrome b_5 and the active portion of its reductase also must be oriented outwardly. Acyl-CoA derivatives 9 to 20 carbons long bind to the enzyme, but derivatives 12 to 19 long carbons are required for desaturase activity. The dependence of the CoA moiety for enzyme recognition and activity is suggested by the decreased activity obtained with dephospho or deamino analogs of stearoyl-CoA. Inhibitor studies with 9,10-substituted acyl-CoA derivatives indicate that carbons 9 and 10 must be in the eclipsed (gauche) conformation at the active site. It was determined that stearoyl-CoA is first integrated into the membrane before it becomes available for the desaturase by mixing stearoyl-CoA with dimyristoyl lecithin liposomes and desaturase-dimyristoyl lecithin liposomes followed by separation by using gel filtration. Even when mixed in the ratio of 20 mol stearoyl-CoA/100 mol lecithin, 56% of the stearoyl-CoA was found in the liposome.

When egg yolk lecithin was used for vesicle formation, SCD activity was twice that when dimyristoyl lecithin was used, possibly because of differences in length of the membrane-forming hydrocarbon tails but, most probably, because of decreased lateral diffusion in a more rigid vesicle when dimyristoyl lecithin was used. This experiment also indicated that *in vivo* differences might exist in desaturase activity based on fatty acid composition of the membranes.

Jeffcoat et al. (1977) studied the properties of rat liver SCD in a crude microsomal system and in a partially purified system. Bovine serum albumin (BSA) and cytoplasmic rat liver preparations increased activity of the crude preparation but did not stimulate activity in the partially purified preparations. It was surmised that BSA acts to protect the acyl-CoA from thioesterases in the crude preparations. Fourteen-, 15-, 16-, 17-, 18-, and 19-carbon acyl-CoA were tested for specificity by the partially purified enzyme and, as was expected from previous work, the order of preference was 18-, 16-, and 19-carbon acyl-CoAs. Based on amino acid analysis of the purified enzyme, SCD was found to be more hydrophobic, and therefore more likely buried deeper in the membrane, than NADH-cytochrome b_5 reductase and cytochrome b_5.

Prasad et al. (1980) demonstrated that the Δ^9-desaturase of chicken liver endoplasmic reticulum was located on the cytosolic face of the membrane by using antibodies specific to the terminal desaturase. Linoleoyl-CoA desaturase was demonstrated to be on the cytosolic side of the endoplasmic reticulum in rat liver as determined by trypsin digestion, antibody inhibition, and using sodium deoxycholate to destroy the permeability barrier of the endoplasmic reticulum (Fujiwara et al., 1984). The other proteins necessary for terminal desaturase activity—NADH-cytochrome b_5 reductase and cytochrome b_5—are integral

membrane proteins that have hydrophilic active sites (De Pierre and Ernster, 1977) and that must be oriented to interact with the active site of the desaturases.

Pollard et al. (1980) screened various CoA-esters to determine the chain-length specificities of Δ^5- and Δ^6-desaturases. Delta-9 *cis*,12 *cis* fatty acids of chain length 14-22 were all substrates for Δ^6-desaturase, ranging from 74% desaturation for the 22-carbon acid to 13% desaturation for 18:2(n-6), the "native" substrate. Delta-5-desaturase is most active with the 20-carbon acids with *cis* double bonds. The Δ^5-desaturase is under much more stringent chain-length control than is the Δ^6-desaturase as demonstrated by 20-, 18-, and 17-carbon Δ^9-fatty acids having 55%, 7.5%, and 3% Δ^6-desaturation, respectively, under *in vitro* conditions.

Fujiwara et al. (1983) confirmed by using a Δ^6-desaturase monospecific antibody that Δ^5-, Δ^6-, and Δ^9-desaturases were definitely different enzymes. Immunodiffusion was used to test the reactivity of the antibody to Δ^6-desaturase in liver microsomes. Then, partially purified Δ^9-desaturase was tested without success. Also, the antibody inhibited Δ^6-desaturase activity, but not Δ^5- or Δ^9-desaturase activity, confirming the uniqueness of the other two desaturase from Δ^6-desaturase.

Several accessory proteins that support fatty acid desaturation in animal cells have been identified. Jones and Gaylor (1979) screened the crude cytosolic fraction from rat liver in search of proteins that stimulated SCD. One such protein with an approximate mass of 26,500 Da was purified 1,100-fold. This unidentified protein stimulated the reaction but did not bind 18:1(n-9) or 18:0, nor did it change the ratio between O_2 consumed per NADH oxidized per stearoyl-CoA desaturated. Because it does not seem to interact with the

substrate or product and does not change the K_m, the authors speculated that this unidentified protein may somehow act to organize the mutlienzyme complex in the membrane. Catalá (1986) describes the properties of a smaller 12 kDa protein partially purified from rat liver that binds 18:1(n-9) preferentially to 18:0. When this protein is added to an *in vitro* Δ^9-desaturase assay, the reaction is stimulated unless the protein is first saturated with 18:1(n-9), in which case, it has no positive effect on the reaction. A lipoprotein-like cytosolic particle has been studied by Leikin and Brenner (1986) that is crucial for optimal Δ^6-desaturase activity. The particle has a protein to lipid ratio of 1.22, with free fatty acids and phosphatidylcholine as its main lipid components. When microsome are washed extensively, this factor is removed along with much of the Δ^6-desaturase activity. Activity can be restored *in vitro* by adding the isolated factor back to the reaction. The factor has been demonstrated to remove the product of the Δ^6-desaturase reaction, 18:3(n-6), in a specific manner and transport it away from the microsome where it could otherwise inhibit the enzyme. The factor does not function by increasing delivery of the substrate to the enzyme because transport occurred equally with and without the factor. Leikin and Brenner provided data supporting a cytosolic factor for Δ^6- (1986) and Δ^5-desaturases (1989) from rat liver microsomes to achieve maximal activity. The Δ^6-desaturase required cytosolic fraction acts by specifically taking up 18:3(n-6), the reaction product, and preventing product inhibition. The Δ^5-desaturase-required cytosolic fraction acts in a similar manner by specifically binding 20:4(n-6) and preventing inhibition. When the cytosolic fraction is preincubated with 20:4(n-6), it is not helpful to the reaction, but, when preincubated with 18:3(n-6), the factor retains its ability to activate the Δ^5-desaturase reaction.

Fifty-fold induction of SCD expression in liver can be achieved by feeding fat-free diets and administering insulin injections (Oshino and Sato, 1972). After the metabolic modifications are stopped, the enzyme activity quickly returns to normal with a half-life of 2 hours (Oshino and Sato, 1972). To better understand SCD half-life, Ozols (1997) studied the degradation of SCD in an attempt to better understand the specificity and rapid loss of desaturase activity after metabolic treatments are changed. Desaturase activity was present in microsomal, nuclear, and subcellular fractions of hepatocytes from rats alternately fasted and refed a low-fat diet. Incubating the fractions at physiological temperature and pH led to complete disappearance of activity. The N-terminal sequence of the desaturase isolated from microsomes was blocked and had three more amino acid residues than did the desaturases found in the nucleus and the subcellular fractions, indicating different processing for the desaturase in the latter two locations. Microsomes washed with high-salt buffer retained activity longer than did those washed with typical buffers. Ozols speculates that the salt may render the enzyme inaccessible to the responsible proteases. A myriad of protease inhibitors including leupeptin, pepstatin, N-acetyl-leucyl-leucyl-methional, phenylmethylsulfonyl fluoride, and lactacystin had no effect in helping retain Δ^9-desaturase activity.

Plant Systems

Stumpf and James (1963) reported ACP in plants and determined that it had a central role in fatty acid biosynthesis much as in *E. coli*. Nagai and Bloch (1968) demonstrated that chloroplasts from spinach elongate ACP-thioesters provided to the reaction and that there is 18:1(n-9) production when O_2, NAD(P)H, NAD(P)H ferredoxin oxidoreductase, and ferredoxin are available. This system described by Nagai and Bloch was the first reported

soluble enzyme system for converting 18:0 to 18:1(n-9). Oilseed-producing plants were, and continue to be, of great interest with respect to SACPD activity because many of them such as safflower have greater than 75% 18:2(n-6), all of which must originate as 18:0 and be converted to 18:1(n-9) by SACPD.

In 1979, Ohlrogge et al. published further proof that fatty acid synthesis in spinach leaf occurs in the chloroplast and is dependent on ACP. Antibodies to spinach ACP inhibited greater than 98% of fatty acid synthesis activity in leaf homogenates. By gently separating the components of the spinach leaf cells by sucrose gradients and by using radioimmunoassay for ACP, all of the ACP was found to be associated with the chloroplast fraction. This paper provided strong evidence that *de novo* fatty acid synthesis occurs exclusively in the chloroplast and that fatty acids must be transported to the cytosol for further modification.

McKeon and Stumpf (1982) purified and characterized the SACPD from maturing seeds of safflower. They were able to purify the enzyme 200-fold and determine that it was a dimer with a molecular weight of 68 kDa. The order of substrate preference was overwhelmingly stearoyl-ACP followed by stearoyl-CoA and palmitoyl-ACP. When comparing stearoyl-CoA to stearoyl-ACP as substrates, the V_{max} values are similar (106 nmoles/min•mg) but the K_m values differ greatly (0.38 μM for stearoyl-ACP and 8.3 μM for stearoyl-CoA). Because of the disparity in K_m values, substrate concentration has much control over enzyme activity. Below the critical micellar concentration of stearoyl-CoA (2 μM), the reaction is first order, but, above this concentration, the graph of activity versus substrate concentration is sigmoidal and plateaus. Catalase increases activity by protecting the system from hydrogen peroxide generated by the aerobic oxidation of ferredoxin and ferredoxin

reductase. When the plant and animal desaturases are compared, they have similar K_m and V_{max} for stearoyl-CoA, but for the plant enzyme to work appreciably well on stearoyl-CoA it has to be above the critical micellar concentration.

Once the properties of the soluble plant fatty acid desaturases were elucidated and their economic and scientific merits were noted, the cloning of a series of desaturases began. Thompson et al. (1991) isolated the cDNA for SACPD from safflower embryos and expressed it in *E. coli*. The enzyme was not active in *E. coli*, but activity was measured in lysate when spinach ferredoxin was added, demonstrating the specific requirement for plant ferredoxin. Since then, many soluble plant fatty acid desaturases have been cloned. More examples of cloned plant enzymes and transgenic applications are presented in the molecular biology section of this review.

Pathways of PUFA synthesis in plants recently was elucidated (Shanklin and Somerville, 1991). The first double bond is formed while 18:0 is esterified to ACP as described above. Beyond the first double bond, further desaturation occurs after the fatty acid has been incorporated into a lipid such as monogalactosyl diacylglycerol or phospholipid. The order of desaturation occurs in the order Δ9, Δ12, and Δ15.

Reaction Chemistry

Experiments elucidating the enzymology of both plant and mammalian desaturases provided insight to the chemistry of the desaturase reaction. Further insight was provided by primary amino acid sequence data and computer modeling of the protein. But, nothing has been more useful in determining the exact mechanism of plant enzymes and projecting mechanisms for mammalian enzymes than has crystallography of the plant desaturases. The

preliminary crystal structure of Δ^9-stearoyl ACP desaturase was the first fatty acid desaturase to be solved by X-ray crystallography (Schneider et al., 1992). A more detailed analysis followed when Lindqvist et al. (1996) published the 2.4 Å crystal structure.

Despite the variability in sequence of membrane-bound desaturases from mammals, fungi, insects, higher plants, and cyanobacteria, all have three regions of conserved primary sequence containing $HX_{(3\text{ or }4)}H$, $HX_{(2\text{ or }3)}HH$, and $HX_{(2\text{ or }3)}HH$ (Shanklin et al., 1994). The histidine-rich regions in the different desaturases are in similar position relative to hydrophobic, membrane-spanning regions of the enzyme. By mutating each histidine residue from a rat cDNA for SCD to alanine, one at a time followed by expression in a Δ^9-desaturase-deficient yeast strain, all eight histidines were found crucial for maintaining active enzyme. It is speculated that the eight histidine residues are critical in maintaining the diiron center of the enzyme. The diiron center places SCD in a class with alkane hydroxylase and xylene monooxygenase as a new class of diiron center-containing enzymes.

Fox et al. (1993) were the first group to study in detail the catalytic core of castor bean SACPD. It was determined that the enzyme contained four atoms of catalytically essential iron that was in a diiron-oxo cluster as determined by Mössbauer spectroscopy. The catalytic core was similar to other enzymes such as methane monooxygenase hydroxylase and ribonucleotide reductase. Furthermore, a set of histidine and carboxylate ligands were postulated to hold the diiron-oxo cluster. Physical chemistry properties of the desaturase when overexpressed in E. coli were studied by using Mössbauer spectroscopy. The results of this work revealed four atoms of iron per homodimer. In the oxidized state, the iron atoms were in the diferric (Fe+3) state but, when reduced, they returned to the diferrous state (Fe+2). Addition of stearoyl-CoA and O_2 caused the iron atoms to return to the diferric state. This catalytically

essential diiron-oxo cluster also has been observed in ribonucleotide reductase and methane monooxygenase hydroxylase. These three enzymes perform extremely different reactions, but all catalyze O_2-dependent cleavage of unactivated C-H bonds, probably via reactive high-valent iron-oxo intermediates. Interestingly, besides the histidine-rich regions, the only sequence commonality between the enzymes is a conserved pair of amino acid sequences –(Asp/Glu)-Glu-Xaa-Arg-His- separated by about 100 amino acids.

Ling et al. (1994) studied the formation of the iron-radical cofactor in the R2 subunit of rionucleotide reductase, an iron-oxo enzyme in the same family as methane monooxygenase and the soluble fatty acid desaturases. By using resonance Raman spectroscopy, it was determined that the diferrous cluster in the reduced form of the enzyme functions as a tyrosine oxidase by using O_2 to oxidize Tyr-122 to a stable radical and form an oxo-bridged diferric cluster. In the mechanism proposed, there is a peroxo intermediate that undergoes heterolytic O-O bond cleavage, resulting in the high valent iron intermediate and water. It is proposed that generation of the high valent intermediate is accomplished by the negatively charged ligands "pushing" and acid-catalysis "pulling" to accomplish the O-O cleavage. The differences within this family of enzymes for electron abstraction or oxygen atom insertion probably lies in slight structural variation, leading to changes in the orientation of the high valent intermediate form of the reactive center.

The 2.4 Å crystal structure of SACPD published by Lindqvist et al. (1996) has provided great insight about the positioning of the catalytic diiron cluster, the point of double bond insertion, and the lipophilic channel that positions the substrate conferring enzyme specificity. The 363 amino acid monomer contains 11 α-helices, nine of which form an antiparallel helical bundle. The two iron molecules of the diiron catalytic site are bound by

pairs of amino acid residues glutamate 196/histidine 232 and glutamate 105/histidine 146. Electron sparcity, corresponding to a μ-oxo bridge, and the 4.2 Å distance between the irons indicate the diferrous form of the enzyme. When the substrate, 18:0, is modeled into the deep channel, the Δ^9-carbon is located in the vicinity of the iron ions. The shape of the Δ^9-desaturase subunit is 35Å X 35Å X 50Å. Except for a small region of β-hairpin loop, the molecule is composed of helical secondary structure. The crystal structure corroborates the classic diiron metal cluster proposed by Fox et al. (1993). Because of the 4.2 Å distance between the irons, the enzyme is probably in the reduced form as a result of X-ray photochemical reduction. The authors propose two possible routes for electron transfer from the surface to the iron center. One scenario utilizes the axis of a helix bundle containing mostly aromatic side chains. The other scenario utilizes a configuration of amino acids analogous to those found in R2 of ribonucleotide reductase. With the catalytic site buried deep within the enzyme, the expected hydrophobic groove to allow acyl chain entry is found. In fact, the channel has a bend at the position of the iron cluster facilitating binding of oleoyl-ACP with its *cis* configuration at the double bond.

Pathways

In 1960, Klenk and Mohrhauer injected ^{14}C-labeled fatty acids into rats and proposed many of the pathways that are now in biochemistry text books for fatty acid desaturation and elongation (Figure 2). The direct desaturation of 22:5(n-3) to 22:6(n-3) by a proposed Δ^4-desaturase as commonly diagrammed, however, was not or has not been demonstrated. Voss et al. (1991) proposed a modification to the previously accepted desaturase/elongase pathways by demonstrating in rat liver microsomes that the desaturation of 22:5(n-3) to

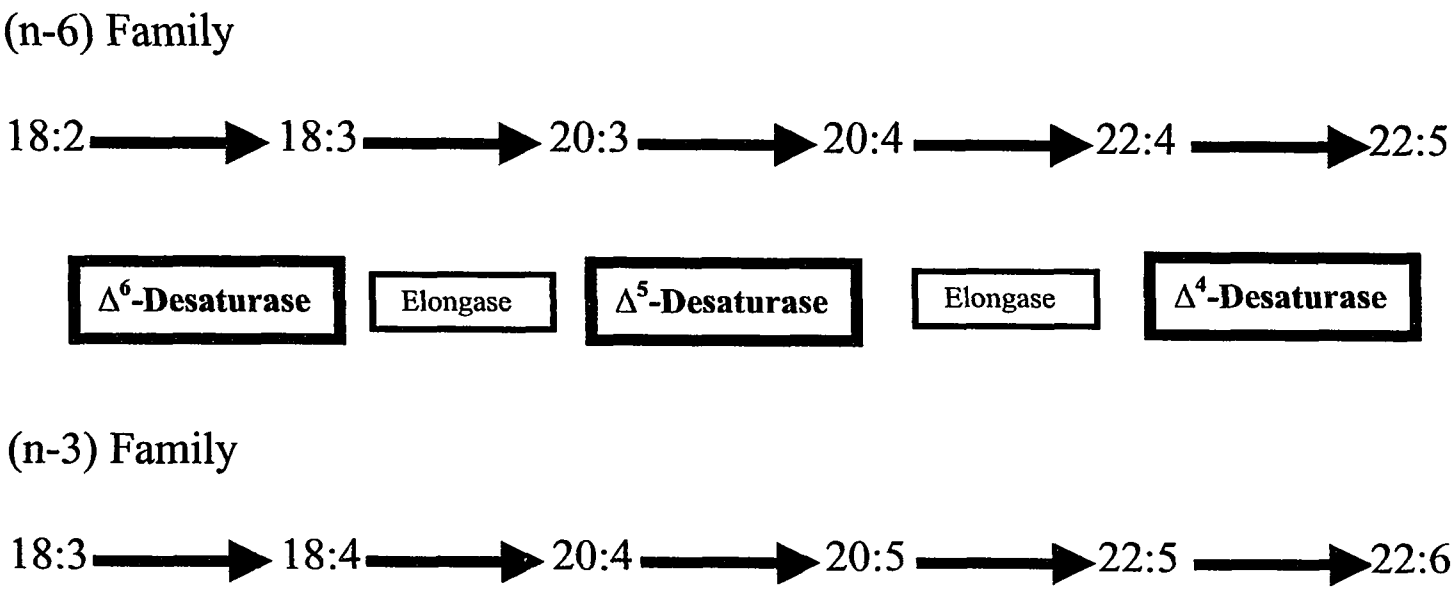


Figure 2. Biosynthesis of the (n-6) and (n-3) families of polyunsaturated fatty acids. Adapted from *Harper's Biochemistry*.

22:6(n-3) was indirect via an elongation to 24:5(n-3), a Δ^6-desaturation to 24:6(n-3), and β-oxidation to 22:6(n-3) rather than a direct Δ^4-desaturation. A parallel scheme was demonstrated by Mohammed et al. (1995), showing that the (n-6) acid 22:4 is converted to 22:5 not via Δ^4-desaturase, but rather by the elongation, Δ^6-desaturation, and β-oxidation to yield 22:5(n-6). Because of the requirement of desaturases, elongases (endoplasmic reticulum), and β-oxidation (peroxisomes), there is an increased amount of trafficking involved in transporting the fatty acids to different areas within the cell during synthesis. A review by Sprecher et al. (1995) discusses alternative pathways for biosynthesis of fatty acids in mammals whereby 24-carbon acids are moved from the endoplasmic reticulum to the peroxisome for partial β-oxidation and then return to the endoplasmic reticulum for further desaturation, bypassing the requirement for direct Δ^4-desaturation.

In the alternative path of 22:6(n-3) production proposed by Voss et al. (1991), Δ^6-desaturase uses 18:3(n-3) as a substrate to produce 18:4(n-3) in what has been considered the typical desaturation scheme, and it uses 24:5(n-3) as a substrate to make 24:6(n-3) in the newly proposed scheme that bypasses the need for a Δ^4-desaturase. Henderson et al. (1998) examined the conversion of 18:3(n-3) to 24:6(n-3) by using trout microsomes as an enzyme source and purified and quantified both labeled and unlabeled intermediates. They concluded that Δ^6-desaturase is feedback inhibited the strongest by the 24-carbon polyunsaturates in the (n-3) desaturation scheme. It also was found that at equal concentrations 24:5(n-3) is the preferred substrate for Δ^6-desaturase over the traditional 18:3(n-3). Furthermore, the 18:3(n-3) desaturation is inhibited by the 24-carbon (n-3) acids, but the 24:5(n-3) desaturation is not inhibited by the 18-carbon (n-3) acids.

The existence of two unique Δ^6-desaturases, one for the traditional and one for the alternate pathway was examined by Geiger et al. (1993). *In vitro* studies utilized rat hepatic microsomes. Homo-gamma-linolenic acid did not inhibit Δ^6-desaturation of either substrate, but each substrate did inhibit the Δ^5-desaturation of 20:3(n-6) to 20:4(n-6). By incubating both the traditional and alternative substrate with microsomes and holding one substrate concentration steady and increasing the other, preferential desaturation of 18:3(n-3) was noted in this system. All data indicate a single desaturase but, until molecular approaches are used, the possibilities of two forms of Δ^6-desaturase exist. On the contrary, Marzo et al. (1996) proposed two different Δ^6-desaturases existed in the conversion of 18:3(n-3) to 22:3(n-6). Two modes of evidence of multiple Δ^6-desaturases are: 1. *Trans*-9,12-18:2 is a potent inhibitor of Δ^6-desaturase and it prevents synthesis of 22:3(n-6) from 18:3(n-3) but not from 20:5(n-3) and 2. When Y-79 cells differentiate, traditional Δ^6-desaturase activity is greatly increased, but the synthesis of 22:6(n-3) remains constant or decreases.

The traditional Δ^6-desaturase reaction was deemed rate limiting by Yamazaki et al. (1992) when they compared the conversion of 18:3(n-3) and 18:4(n-4) to 20:5(n-3). After feeding rats 10% lard or 9% lard supplemented with 1% test fatty acid as an ethyl ester for one or 3 weeks, liver and blood fatty acids were identified. Delta-6-desaturase was determined to be the rate-limiting step in the desaturase/elongase scheme because rats fed 18:4(n-3) had 2-fold increases in 20:5(n-3) accumulation in hepatic and blood lipids compared with the 18:3(n-3)-fed rats. Also, 18:4(n-3) was not detected in most fractions tested, even in rats eating diets containing 1% 18:4(n-3).

Control Mechanisms of Desaturase Activity in Animal Systems

Diet

Fat

Introduction

The effects of dietary fatty acids on metabolic regulation was first noted by Allmann and Gibson (1965) when they noticed a 70% depression in hepatic fatty acid synthesis, malic enzyme activity, and glucose-6-phosphate dehydrogenase activity 2 days after feeding only 2% 18:2(n-6) in an otherwise fat-free, high-carbohydrate diet. While dietary fats act by both controlling cellular metabolism by changing fatty acid composition of membranes and regulating nuclear events that govern transcription (review by Clarke and Jump, 1996b), the rapid suppression of enzyme systems is a transcriptional event (Blake and Clarke, 1990). Transcriptional down-regulation occurs within 3 hours after feeding diets containing 5% menhaden oil to animals adapted to a high-carbohydrate, fat-free diet (Clarke et al., 1990). Clark and Jump (1993a) have demonstrated that depression of lipogenic enzyme expression occurs with (n-6) and (n-3) fatty acid intakes at three to four times the amount required for optimal growth. Transcription returns to normal within 3 hours after removing the oil from the diet. While membrane fatty acid composition has been recognized to influence the cell at the molecular level by altering such things as hormone signaling (Spector and York, 1985), these data emphasize the probability that PUFA is exerting a direct effect on transcription rather than acting by changing biophysical properties of membranes.

Clarke and Jump have focused on hepatic gene expression, but, more recently, Tebbey et al. (1994a) and Tebbey and Buttke (1992a) identified PUFA inhibition of transcription in lymphoma and adipose cells. By using chimeric promoter/reporter studies,

Jump et al. (1993) studied the 4,000 bp sequences upstream of both S14 and pyruvate kinase genes. The constructs were responsive to PUFA addition to the culture. Then, by doing deletion studies, the regions –220 to –80 and –197 to –96 of S14 and pyruvate kinase genes respectively, contained the PUFA-response region, the same region responsible for the insulin-carbohydrate induction response. Because of the rapidity of PUFA response, scientists have been looking for a nuclear factor that would mediate the PUFA signal. The first example of this type of factor came in the cloning of the PPAR that responds to, among other things, fatty acids (Gearing et al., 1993). But, as Clarke and Jump (1996a) point out in their review, not all genes that respond to PUFA have a PPAR-response element, nor does PPAR discriminate between fatty acids as is seen with PUFA, but not 18:0 for example, repression of transcription.

While examples of fatty acids other than PUFAs acting to modulate desaturation are sparse in the literature, there are a few examples. Methyl branched-chain fatty acids (MBCFA) inhibit SCD activity by over 50% in rat liver when fed at 50 mg/g diet (Wahle and Hare, 1980). Branched-chain fatty acids are found in the adipose tissue of ruminants and accumulate as methylmalonyl-CoA, an intermediate in propionate metabolism that is incorporated into fatty acids. The inhibition by MBCFA seems specific and not a general membrane effect because other membrane-dependent reactions are not affected (ω-hydroxylation) and because MBCFA inhibit *in vitro* reactions, whereas palmitate does not.

Periago et al. (1989) fed rats olive oil or corn oil with and without supplemental medium chain triacylglycerols (MCT) to study the effects on microsomal membrane fatty acid composition and desaturase activities. The corn oil-fed rats had more saturated fatty acids in the microsomal membranes than did olive oil-fed rats. While this cannot be

explained on the basis of dietary fatty acids directly, it is now understood that the increased 18:2(n-6) in corn oil depressed Δ^9-desaturase activity and that this inhibition led to an increased saturated fatty acid content of the membranes. Medium chain triacylglycerols depressed Δ^9-desaturase when fed with olive or corn oil but only suppressed Δ^6-desaturase when fed with corn oil. The mechanism for the disparity between Δ^6- and Δ^9-desaturase activities when oils are fed in combination with MCT is not known, however it could be an indirect effect via the changes in insulin and glucagon status as a result of the dietary MCT.

Dietary Polyunsaturated Fatty Acids

The early studies of PUFA regulation of fatty acid desaturases measured enzyme activities as a response to dietary fat. Pugh and Kates (1984) studied the activities of hepatic fatty acid desaturases and 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase in rats fed chow alone or with added corn oil or coconut oil. Whereas Δ^9-, Δ^6-, and Δ^5-desaturases were increased with corn oil and decreased with coconut oil relative to chow-fed rats, there was no difference in 1-acyl-*sn*-glycero-3-phosphocholine acyltransferase activity with these dietary treatments. The fatty acid composition of hepatic microsomes did not correlate with the *in vitro* determined activity of the desaturases possibly because of dietary fatty acids being incorporated directly into membrane lipids. These results differ from those of most experiments in that one would expect Δ^9-desaturase to be down-regulated when corn oil is fed. The Δ^5-desaturase results compare with those of Gomez-Dumm et al. (1983) who supplemented fat-free diets with 18:2(n-6), 18:3(n-6), or 20:4(n-6) to study the response of Δ^5-desaturase activity. Fat-free diets depressed Δ^5-desaturase activity by about 50% whereas

feeding any of the (n-6) acids increased Δ^5-desaturase activity. The increase in Δ^5-desaturase is probably because of increased enzyme synthesis, as cycloheximide blocked the reactivation. Gamma-linolenic and 20:4(n-6) showed the most potent response with recovery of Δ^5-desaturase activity to control values within 48 hours after feeding.

de Antueno et al. (1993) demonstrated a change in plasma 18:1(n-9) to 18:0 ratio when mice were fed 2.5% (n-3)-, (n-6)-, or hydrogenated coconut oil-enriched diets. Because the diets had similar ratios of 18:1(n-9) to 18:0 and because liver Δ^9-desaturase activity correlated positively with plasma 18:1(n-9) to18:0 ratios, hepatic Δ^9-desaturase activity can be estimated by monitoring plasma fatty acid composition. Estimates of Δ^6-desaturase activity made by monitoring the ratio of total (n-3) to 18:3(n-3) or total (n-6) to 18:2(n-6) in plasma indicate that Δ^6- and Δ^9-desaturase activities are correlated positively when the different dietary fatty acids were fed to induce changes in lipid metabolism. The authors also concluded that absolute PUFA concentrations or PUFA to saturated fatty acid ratios were not major factors in determining plasma triacylglycerol concentration or Δ^9-desaturase activity. However, dietary 20:5(n-3) had a major effect on plasma triacylglycerol concentration and Δ^9-desaturase activity only when fed in combination with 18:3(n-6). This study conforms with other studies showing that dietary (n-3) fatty acids are correlated inversely with plasma triacylglyceride concentrations and Δ^9-desaturase activity probably via an insulin or insulin receptor interruption.

The (n-6) fatty acid suppressing effect on adipose Δ^9-desaturase was studied by Kouba and Mourot (1998) by feeding pigs diets enriched in 18:2(n-6) (4% corn oil) or 18:0 (4% beef tallow) compared with a conventional low-fat swine diet. The 18:2(n-6)

enrichment augmented lipogenesis by increasing the activities of acetyl-CoA-carboxylase, malic enzyme, and glucose-6-phosphate dehydrogenase. But because of increased PUFA in the 18:2(n-6)-enriched diet, Δ^9-desaturase activity was decreased compared with that in pigs fed the other two diets. The tissue fatty acid composition reflected the changes in Δ^9-desaturase activity.

Essential Fatty Acids

Lefkowith (1990) studied the rate of development of essential fatty acid deficiency, typically a difficult scenario to induce experimentally, when Δ^9-desaturase was induced. The author suspected that by increasing Δ^9-desaturase activity he would observe a great increase in 20:3(n-9), the hallmark fatty acid that accumulates during fatty acid deficiency. Being able to speed the induction of essential fatty acid deficiency would be of great value in the time savings it would offer in experimental models. Whereas induction of Δ^9-desaturase by alternately fasting and feeding a fat-free diet increased hepatic 20:3(n-3) to 20:4(n-6) ratios dramatically, the other samples such as plasma, macrophages, and renal cortex changed only slightly or not at all. From this research, it can be noted that essential fatty acid deficiency is resisted strongly by the body and that the liver has a pivotal role in maintaining PUFA status of the organism.

Up-regulation of Δ^5- and Δ^6-desaurases in HepG2 cells grown with normal and essential fatty acid-deficient (EFAD) media was studied by Melin and Nilsson (1997). When ^{14}C-18:2(n-6) was provided in the media, EFAD cells converted six times more radioactivity into desaturase and elongase metabolites than did control cells. There was 14 times more

labeled 20:4(n-6) in EFAD cells than in controls, indicating the potential for up-regulation of both Δ^5- and Δ^6-desaturases and their limited expression in HepG2 cells fed essential fatty acids.

Bond Configuration

The control of Δ^9-desaturase activity as influenced by the configuration of double bonds in 9,12-dienoic fatty acid structures was studied by de Alaniz et al. (1986). Various fatty acids were supplied for 24 or 48 hours to rats previously maintained on a fat-free diet. The configuration of the bonds proved to be critical as 18:2(9 *trans*,12 *trans*) did not change Δ^9-desaturase activity at all compared with the fat-free fed rats. Linoleic acid, 18:3(n-6), and 18:3Δ(5t,9c,12c) all suppressed Δ^9-desaturase activity with increasing potency. Presumably, Δ^9-desaturase repression is elicited by metabolites of 18:2(n-6) and 18:3(n-6), as 18:1(n-9 *trans*) elaidic acid cannot be metabolized further. Because columbinic acid (18:3(5 *trans, 9 trans,* 12 *cis)*) is not a substrate for Δ^6-desaturation and because the space-filling structure is similar to 18:2(n-6), the importance of the 9-*cis*, 12-*cis* configuration in regulation of Δ^9-desaturase expression is confirmed.

Clarke and Jump (1993b) described the physical configurations of PUFAs to act as gene regulators. The requirements established so far include two double bonds in an 18-carbon fatty acid with one bond positioned between carbons 9 and 10 and the other between 12 and 13. One of the bonds must be in the cis configuration, but the other can be in the trans configuration.

Conjugated linoleic acid (CLA) does not fit into the prescribed bond configurations for gene regulation, but it does cause a decrease in SCD type one (SCD1) activity in the liver of mice (Lee et al., 1998a). When mice were fed a fat-free diet or a diet with 5% corn oil, CLA addition at 0.5% caused a 45% and 75% decrease in SCD1 mRNA, respectively. Conjugated linoleic acid at 150 μM in the media of cultured hepatocytes suppressed SCD1 expression by 60%. Authors note that it is not the 9 *cis*, 11 *trans* isomer but probably the 10 *trans*, 12 *cis* of CLA that is responsible. In initial studies, it was noted that CLA was about twice as potent as 20:4(n-6) at down-regulating SCD1 expression.

Polyunsaturated Fatty Acids/Insulin Interaction

Ntambi et al. (1996) used a mouse liver cell line (H2.35) to study insulin and PUFA effects on SCD1 expression. Insulin stimulated expression whereas PUFAs down-regulated expression. Polyunsaturated fatty acids were able to suppress the insulin induction, with 20:4(n-6) being the strongest suppressor. Fusions of the 5' regulatory sequence for SCD1 were made with the chloramphenicol-acyltransferase (CAT) cDNA and expressed in H2.35 cells. Both insulin and PUFAs were able to modulate expression of CAT, indicating the control region for both of these modulators is in the 5' promoter region.

Polyunsaturated fatty acids decrease mouse liver SCD1 activity through an insulin-independent mechanism as demonstrated by Waters and Ntambi (1996). When diabetic mice were provided PUFA plus insulin or PUFA plus fructose, lipogenic gene expression is up-regulated in the presence of increased carbohydrate or insulin and down-regulated in the presence of PUFAs. The regulatory sequences upstream of the S_{14} and pyruvate kinase genes have been studied, and the regions that are required for PUFA inactivation are the same

regions required for insulin/carbohydrate induction (Thompson and Towle, 1990). The apparent dual functionality of upstream regulatory sequiences quickly leads to speculation about PUFA interfering with the insulin signaling pathways.

Polyunsaturated Fatty Acids/Cholesterol Interaction

Garg et al. (1988a,b,c) published a series of articles reporting the regulation of fatty acid desaturases after feeding fish oil linseed oil, and beef tallow to rats. The fish oil, and linseed oil diets depressed Δ^9-desaturase activity by 90% and 60%, respectively, compared with the beef tallow feeding. Interestingly, cholesterol addition stimulated the Δ^9-desaturation of 16:0, resulting in increased concentrations of 16:1(n-7) and 18:1(n-7). In summary, it is evident that Δ^9-desaturase is dependent not only on the fatty acid environment but also the cholesterol environment of the membrane. It can be concluded that monounsaturated fatty acid (MUFA) synthesis is not regulated by content of MUFAs in the diet because dietary MUFA was correlated positively with Δ^9-desaturase activity in this study. The authors concluded that increased membrane fluidity caused via feeding PUFAs, or less cholesterol, is the primary mechanism for Δ^9-desaturase regulation. Inhibition of Δ^6-desaturase activity was greater in the fish oil-fed rats than in the linseed oil-fed rats. Cholesterol only inhibited Δ^6-desaturase activity when fed with tallow, but, in combination with the concentrated (n-3) diets, it had no effect on desaturase activity. This observation was one of the first to explain a biochemical reason for the decrease in prostaglandin E_2, thromboxane, and prostacyclin concentrations after feeding fish oil. Also, small amounts of dietary fish oil block the cholesterol inhibition of Δ^6-desaturase when a diet rich in saturated

fat is consumed. Fish oil suppressed Δ^5-desaturase relative to beef tallow whereas linseed oil increased Δ^5-desaturase relative to tallow. Cholesterol added to the beef tallow and linseed oil-enriched diets casued a decrease in Δ^5-desaturase, but, cholesterol added to the fish oil-enriched diet did not change Δ^5-desaturase activity. Fatty acid composition of liver microsomes were predictable based on the *in vitro* desaturase activity data.

Muriana et al. (1992) fed rats diets containing 10% olive oil or fish oil with and without 1.0% added cholesterol. Fish oil feeding increased Δ^9-desaturase and decreased Δ^5- and Δ^6-desaturase activities relative to that of olive oil-fed rats. Adding cholesterol increased Δ^9-desaturase with both oils and decreased Δ^6-desaturase activity with both oils. Adding cholesterol increased Δ^5-desaturase activity with fish oil and decreased activity with olive oil. Patterns in fatty acid composition of liver microsomal membranes matched fatty acid desaturase activity data. This report shows the extreme modulation of desaturases with dietary modifications in rats.

Polyunsaturated Fatty Acids and Gene Regulation

There are many good review articles that have been written in the past few years on dietary PUFA and gene regulation. One of the most recent is a review by Sessler and Ntambi (1998), that provided a thorough review of the effects of PUFAs on gene expression. Whereas PUFAs are important in membrane structure, metabolism, and signal transduction, it has become clear that PUFAs also have a role in the control of adipogenesis and physiologic events that regulate expression of enzymes crucial to energy metabolism. Hepatic-specific PUFA regulation was reviewed by Clarke and Jump (1996a,b).

Polyunsaturated fatty acids exert their effect in some cases through eicosanoid production, but, in the case of lipogenic enzyme regulation, the effect is independent of eicosanoids. The PPAR has been proposed by some to be the PUFA-binding trans acting factor responsible for down-regulating lipogenic gene expression (Gearing et al., 1993), but there is evidence to support a more direct PUFA effect on gene transcription. S_{14} and pyruvate kinase promoter regions do not contain a PPAR-response element (Jump et al., 1995). Transcription trans-activating assays all give equal response to saturated fatty acids, MUFAs, and PUFAs (Gottlicher et al., 1992)

Clarke and Jump (1993b) also wrote a review on regulation of gene transcription by PUFAs. The decrease in hepatic lipogenesis by (n-3) or (n-6) acids seems to be specific to liver tissue, as adipose (Clarke et al., 1977) and lung (Clarke et al., 1984) lipogenesis was not selectively suppressed by (n-6) or (n-3) fatty acids. When hepatic fatty acid synthesis is inhibited, triacylglycerol synthesis and secretion (Phillipson et al., 1985) and Δ^9-desaturase (Ntambi, 1992) also are inhibited. The authors of the review speculate that the dramatic effect that PUFAs have is not simply to lower serum triacylglycerol concentrations but rather to allow synthesis of healthy membranes rich in PUFA rather than 18:1(n-9). Monounsaturates would out-compete the PUFAs in phospholipid synthesis if their Δ^9-desaturase was not down-regulated (Gomez-Dumm et al., 1983).

Hepatic SCD1 was grouped with fatty acid synthase and S_{14} in the way that it is down-regulated in response to unsaturated fatty acids (Landschulz et al., 1994). Both in mice and in cultured hepatocytes, fat-free or 18:1(n-9) feeding did not decrease SCD1 mRNA, concentrations, but 18:2(n-6), 18:3(n-3), or 20:4(n-6) caused a significant decrease (up to 90%) in SCD1 mRNA with 20:4(n-6) causing the greatest down-regulation. Run-on

transcription assays indicate that unsaturated fatty acids control mRNA concentration by decreasing the rate of transcription.

Regulation of SCD type two (SCD2) expression in lymphocytes involves positive and negative regulation much like SCD1 in liver (Tebbey and Buttke, 1992b). Arachidonic acid in particular was shown to regulate SCD2 expression primarily by a decrease in transcription. A more detailed investigation of regulation of SCD2 by 20:4(n-6) was provided by Tebbey et al. (1994b). When lymphocytes were grown in 25μM 20:4(n-6), phospholipid 20:4(n-6) increased to 18 wt% and SCD2 gene expression was inhibited. When 20:4(n-6) was removed from the media, phospholipid 20:4(n-6) decreases to 0.6 wt% in 144 hours, but unsaturated fatty acid synthesis is fully operational again within 6-12 hours because of an increase in SCD2-specific transcription and translation. As unsaturated fatty acid synthesis is re-established, the 20:4(n-6) content of phoshatidylcholine declines whereas there is no change in 20:4(n-6) content of phosphatidylethanolamine and phosphatidylserine. The authors suggest a phospholipid intermediate being involved in the signaling pathway whereby dietary fatty acids regulate expression of critical biosynthetic genes.

Dietary trans Fatty Acids

In 1984, Mahfouz et al. published a study where rats were fed high-oleic safflower oil, hydrogenated soy oil, lard, or corn oil to determine dietary fat effects on Δ^6-desaturase activity. Fatty acid composition of the diets was reflected in the fatty acid composition of the hepatic microsomes. The hydrogenated soy oil diet depressed Δ^6-desaturase compared with all other diets, but the other diets did not differ from one another with respect to Δ^6-desaturase activity. This observation indicates that *trans* fatty acids are stronger inhibitors of

Δ^6-desaturase than are saturated fatty acids. Delta-9-desaturase activity was greatest in the high-oleic safflower oil-fed rats and different from that in the corn oil-fed rats that had the least Δ^9-desaturase activity. The lard- and the hydrogenated soy oil-fed rats were intermediate in Δ^9-desturase activity and did not differ from the other treatments. Delta-5-desaturase activity was not changed by any dietary treatments imposed.

The effects of *trans* fatty acids on Δ^5-, Δ^6-, and Δ^9-desaturases also were assessed in rat liver microsomes from rats fed varying amounts of *trans* fatty acids (Mahfouz, 1981). When 20% of the diet was margarine, Δ^6- and Δ^9-desaturases were significantly inhibited, but, when hydrogenated coconut oil was fed, only Δ^9-desaturase was decreased compared with that of control rats. This study demonstrates how dietary *trans* fatty acid could cause effects similar to those of essential fatty acid deficiency via fatty acid desaturase inhibition, leading to inhibition of eicosanoid formation.

Svensson (1983) fed hydrogenated peanut oil and hydrogenated marine oils to rats in a diet normalized for 18:2(n-6) content to study changes in fatty acid incorporation and fatty acid desaturase activity of microsomal membranes. The effects of feeding hydrogenated herring oil was more dramatic on inhibiting all three fatty acid desaturases than diets that were more enriched in *trans* fatty acids, probably because of the combination of *trans* isomers and the long-chain highly unsaturated acids in partially hydrogenated fish oil. Despite decreasing all three desaturases by nearly 50% and inducing dramatic changes in phospholipid fatty acid composition, the authors comment that it is not known if these affects are dramatic enough to affect membrane fluidity or eicosanoid production.

Natural and hydrogenated peanut and marine oils were fed to rats by Kirstein et al. (1983) to delineate the effect of *trans* isomers and acyl-chain length of MUFAs on Δ^5- and Δ^6-desaturase activities. Partial hydrogenation of marine lipids inhibit desaturase activities more than peanut oil, again pointing the finger at the higher concentrations of 20:1 and 22:1 isomers in hydrogenated marine oil

De Schrijver and Privett (1981) fed *trans* fatty acids to fatty acid-deficient and healthy rats. In all cases, *trans* fatty acids depressed Δ^6-desaturase activity *in vitro* and *in vivo* as 18:2(n-6) accumulated when *trans* fatty acids were fed. The Δ^6-desaturase depression was more evident in rats that previously had been maintained on an essential fatty acid-deficient diet. Delta-9-desaturase activity was not affected by *trans* fatty acids and, in fact, was increased when *trans* fatty acids were fed in a balanced diet.

In vitro tissue culture experiments by Rosenthal and Doloresco (1984) used human skin fibroblasts to study the effects of different *trans* isomers of fatty acids on the activity of Δ^5-desaturase. The overall goal was to identify potential specific inhibitors of 20:4(n-6) synthesis in human cells to decrease inflammatory prostaglandin production. The order of potency in inhibiting the Δ^5-desaturase reaction was 18:1(9 *trans*) > 18:2(9 *trans*, 12 *trans*) > 18:1(9 *cis*) = 18:2(9 *cis*, 12 *cis*) > 18:1(11 *trans*) . In summary, it was noted that n-9 *trans* fatty acids are potent inhibitors of Δ^5-desaturase, whereas n-7 isomers are not.

Protein

When Narce et al. (1992) fed rats a low-protein diet for as little as 2 days, Δ^9-desaturase activity inhibition was noted. The enzyme activity was further suppressed 14 days

after feeding a low-protein diet and then rebounded to values greater than control rats when the rats were refed a protein adequate diet. Interestingly, the fatty acid composition of the liver did not represent the activity of the desaturase because 18:1(n-9) concentrations increased in the protein-deficient rats. The only plausible explanation for this discrepancy would be a decreased oxidation or altered transport of fatty acids during the protein-deficient state to conserve membrane integrity of the liver when Δ^9-desaturase is lacking. The decreased ratio of 18:0 to 18:1(n-9) could be explained partially by a decrease in *de novo* synthesis because of the protein-deficient state.

Narce et al. (1988) also studied the effects of protein malnutrition on Δ^5- and Δ^6-desaturase activities in growing rats. Oleic acid, 18:2(n-6), and 18:3(n-3) accumulate in total liver lipids of protein-deficient rats whereas there is a decrease in 20:3(n-6) and 20:4(n-6) when compared with protein-adequate rats, but microsomal phospholipid changes were not observed until 52 days of protein malnutrition. Both desaturases decreased by 20-30% of their original values after rats were fed a low-protein diet for only 2 days. When the low-protein diet was fed for 52 days or when the rats were fed a protein balanced diet, desaturase activities were greatly restored within 2 days and were back to control values after 13 days of refeeding. Liver protein synthesis was impaired because of an increase in fatty liver, an indicator of insufficient apoprotein synthesis to support VLDL export from the liver.

Changes in rat pup fatty acid desaturases as a result of feeding their mothers essential fatty acid- or protein-deficient diets were analyzed by De Tomas et al. (1980). Low-protein diets when fed to the dams decreased pup Δ^5-, Δ^6-, and Δ^9-desaturase activities by about 60%. The low fat diet only changed Δ^9-desaturase; it increased activity by 360%. These results

explain the reason for increased 20:3(n-9) in essential fatty acid deficiency and the decrease in 20:4(n-6) when animals are not receiving an adequate amount of dietary protein.

Specific dietary proteins seem to differentially affect fatty acid desaturases as dramatically as do protein-deficient diets. When highly purified soybean protein or casein are fed to rats, the soy protein feeding results in lower microsomal Δ^6-desaturase activity on (n-6) fatty acids (Madani et al., 1998). Soy protein only lowered Δ^6-desaturase on (n-3) acids when low substrate concentrations (60 nM) were used. The authors suggest this is support for two different Δ^6-desaturases as others have proposed. Delta-5-desaturase, however, was not affected by type of dietary protein. When the diets contained supplemental cholesterol, both desaturase activities were decreased, but Δ^5-desaturase activity decreased more than did Δ^6-desaturase. The cholesterol-induced changes often are thought to be associated with decreases in membrane fluidity.

Lindholm et al. (1993) also fed casein and soybean proteins to rats. The casein-fed rats had greater Δ^6-desaturase activity than did the soybean protein-fed rats. When microsomal fatty acid composition of the two groups of rats was compared, the soybean protein-fed rats had greater concentrations of 18:2(n-6) and 20:4(n-6). The increase in 18 carbon acids can be attributed directly to decreased desaturase activity, whereas the increase in 20:4(n-6) only can be explained by a differential use of that particular fatty acid by rats fed the two diets. The higher concentration of 20:3(n-9) in the casein-fed rats may occur because the increase of Δ^6-desaturase allowed 18:1(n-9) to be more readily utilized as a substrate for the Δ^6-desaturase reaction, leading to increased 20:3(n-9) as is observed in essential fatty acid-deficient animals.

Dietary casein also was shown to enhance the activity of Δ^6-desaturase relative to dietary soy protein in rat liver microsomes because of changes in microsomal membrane fluidity (Koba et al., 1993). When a variety of protein sources were fed that modulated membrane fluidity, a positive correlation ($r=0.804$; $0.05<P<0.1$) was observed between Δ^6-desaturase activity and fluorescence anisotropy (decreased anisotropy means increased fluidity), a measure of membrane fluidity.

Peluffo et al. (1984) studied the effects of differing amino acid diets on Δ^5-, Δ^6-, and Δ^9-desaturase activities. A 45% casein diet decreased Δ^9-desaturase and increased Δ^5- and Δ^6-desaturase activities relative to a typical diet with 5% casein. An amino acid mixture equivalent to that found in casein resulted in no differences compared with the 45% casein diet. By excluding different amino acids from the simulated casein mixture, it was determined that tyrosine and phenylalanine inhibit Δ^6-desaturase, but little change was seen in Δ^5- and Δ^9-desaturase activities when amino acid deletions or substitutions in the diet were made. When massive doses (20% of dietary calories) of phenylalanine, tyrosine, and tryptophan were given, tyrosine inhibited Δ^6-desaturase to 33% of control values, phenylalanine inhibited to 66% of control values, and tryptophan had no effect. The authors speculate that epinephrine formation from phenylalanine and tyrosine could raise cAMP concentrations, which inhibit Δ^6-desaturase activity (Gómez-Dumm, 1975).

Carbohydrate

The extreme induction of hepatic Δ^9-desaturase noted when rodents are alternately fasted and fed a low-fat, high-carbohydrate diet was utilized by Thiede and Strittmatter

(1985) to isolate SCD mRNA from in rat liver. By using northern analysis, a 4,900-base mRNA increased in concentration 50-fold by the dietary induction protocol. Protein synthesis from the recovered mRNA template in a rabbit reticulocyte lysate system detected an immunologically active protein that was only detected at the molecular weight of the isolated enzyme, indicating it is not synthesized in a larger pre-enzyme form.

Ntambi et al. (1992) described the time course of induction of mouse liver SCD1 mRNA when fasted mice were fed a high-carbohydrate, fat-free diet. Induction of SCD1 mRNA was 2-fold within 6 hours of refeeding a fat-free diet and 45-fold within 36 hours of refeeding a fat-free diet. Nuclear run-on assays indicated an increase in transcription as a result of the dietary change to carbohydrate. The half-life of the mRNA, when the mice were switched to a normal chow diet, was about 4 hours. Also, 5% dietary PUFAs, in the form of triacylglycerols, severely decreased the abundance of SCD1 mRNA, whereas MUFA or saturated fatty acids had little or no effect. The effects observed for SCD1 mRNA induction with fat-free feeding are much slower than those of other lipogenic genes such as S14 and fatty acid synthase, indicating a possible unique induction mechanism for SCD1 compared to other lipogenic enzymes.

Vitamins and Minerals

Fatty acid desaturase systems of zinc-deficient rats fed a "saturated" fat diet (coconut oil) or "n-3" diet (linseed oil) were studied by Eder and Kirchgessner (1995). Zinc deficiency disturbs the Δ^9-desaturase system more than the other desaturases, causing a significant decrease in the rats fed a more saturated diet, but no change was observed in rats fed the linseed oil diet. Delta-6-desaturase activity was not changed when 18:2(n-6) was

used as a substrate *in vitro* but was depressed in zinc-deficient rats fed the saturated diet when 18:3(n-3) was used as a substrate. This result is interesting, demonstrating how zinc deficiency can change the affinity of an enzyme, SCD, for a given substrate. Because the rats fed the more saturated diet had ample 18:2(n-6) and the rats fed linseed oil had ample 18:3(n-3), the net *in vivo* rate of Δ^6-desaturase was probably not affected. There were no significant differences in Δ^5-desaturase activity. Despite the differences observed, the action of zinc on "stabilizing" the membrane is probably not via fatty acid desaturase modulation. The similarities between essential fatty acid deficiency and zinc deficiency are not mediated by changes in desaturation of 18:2(n-6) or 18:3(n-3). The impairment of prostaglandin synthesis in zinc deficiency, however, must be mediated in reaction steps between 20:4(n-6) and the prostaglandins.

Zinc is required for efficient desaturation and elongation of the essential fatty acids in rats (Clejan et al., 1982). Cadmium, which is toxic, seems to exert part of its negative effect by antagonizing zinc and inhibiting those biological processes that require zinc. Kudo et al. (1991) demonstrated that rats fed a fat-free diet with supplemental zinc were protected against cadmium inhibition of SCD activity (35% decrease in activity) whereas zinc-deficient animals were not (90% decrease in activity). Fatty acid composition of liver membranes were not changed in zinc-replete rats, whereas 18:1(n-9) and 20:3(n-9) decreased in zinc-deficient rats, supporting the results from *in vitro* enzyme assays. To understand how direct the cadmium inhibition of SCD was, studies were conducted in rat hepatocytes in culture that had been maintained in an EFAD state (Kudo and Waku, 1996). From this experiment, the inhibition seems specific and not mediated by changes in hormonal status (cells cultured in serum free media), as Δ^9-desaturase was inhibited by cadmium and Δ^6-desaturase was

slightly elevated. The inhibition, however, cannot be by direct enzyme inhibition, becuase cadmium does not inhibit *in vitro* Δ^9-desaturase activity.

Low concentrations (30-50 μM) of coupric ions added to *in vitro* assays of SCD, however, inhibits the conversion of 18:0 to 18:1(n-9) primarily by inhibiting the terminal desaturase (Sreekrishna and Joshi, 1980). While cytochrome b_5 and its reductase were inhibited by about 20%, this inhibition does not account for the >80% inhibition of the terminal desaturase. Cupric ion chelators reverse the inhibition. The authors speculate that cupric ions are acting as superoxide scavengers, minimizing the availability of the highly energetic diiron-oxo form of the desaturase.

Rao et al. (1983) fed rats iron-deficient diets with (a) no added fat, (b) with 14% corn oil, or (c) 14% hydrogenated coconut oil and studied SCD activity. Iron depletion significantly decreased the activity of SCD in all three dietary treatments from 1.5- to 2.5-fold. There was down-regulation of SCD1 activity with fat feeding and even more so with polyunsaturated fat feeding in rats fed both iron-deficient and iron-adequate diets. The only rats that displayed clinical signs of iron deficiency (decreased hemoglobin and hematocrit) were the PUFA-fed rats on an iron deficient diet. No known mechanism explains the sparing effect of low-fat or saturated fat feeding on iron status. It is interesting to note that SCD1 activity is an early predictor of marginal iron status even before clinical signs of iron deficiency are detected.

Hepatic microsomes from rats fed either supplemental β-carotene (0 or 100 mg) or supplemental 13-*cis*-retinoic acid (20 or 100 mg) were assayed for fatty acid desaturase activity and microsomal membrane fatty acid composition (Alam et al., 1984). Delta-9-desaturase activity for the control and the 20 mg/kg 13-*cis*-retinoic acid groups were the

same, whereas the groups fed 100 mg/kg of either β-carotene or 13-*cis*-retinoic acid were decreased to about 50% of control values. The Δ^6-desaturase activity was not changed by β-carotene but was increased 70% by 20 mg/kg of dietary 13-*cis*-retinoic acid and almost 200% by 100 mg/kg of dietary 13-*cis*-retinoic acid. The changes of *in vitro* desaturase activity paralleled the increased concentration of unsaturated fatty acids of hepatic microsomes.

Alam and Alam (1985) measured Δ^6- and Δ^9-desaturase activities in vitamin A-deficient rats. Deficient rats had increased Δ^9-desaturase activity *in vitro* which then decreased to near control activity when the rats were repleted. Delta-6-desaturase activity did not change compared with control values during deficiency or repletion.

Retinoic acid, when administered to 3T3-L1 preadipocytes along with the differentiation cocktail (methylisobutylxanthine, dexamethasone, and insulin) used to stimulate lipid accumulation, inhibits transcription of adipocyte lipid-binding protein and SCD1 (Stone and Bernlohr, 1990). The inhibition was specific for differentiation-dependent transcripts, as β-tubulin or glutamine synthase transcription rates were not changed. The "window" for retinoic acid being effective is narrow and the effects are persistent, even if retinoic acid is removed from the media as the cells are allowed to mature.

DL-α-Tochpherol addition to microsomal preparations decreased the activity of Δ^6-desaturase prepared from rat liver, but increased the activity in preparations from rat brain (Despret et al., 1992). The maximal increase in activity corresponded with the minimal activity in liver, occurring at a vitamin E enrichment that was 20-fold greater than the endogenous vitamin E in the microsomes. This is one experiment that may raise questions about humans consuming pharmacological doses of vitamin E.

Ethanol

Chronic ethanol administration decreases SCD activity in rat liver microsomes by greater than 80% (Umeki et al., 1984). During ethanol dosing, NADPH-cytochrome c reductase, cytochrome b_5, and cytochrome P_{450} respond with an adaptive induction, whereas the NADH to NAD ratio of the cell doubles. These data suggest that it is a decrease in the terminal desaturase that results in decreased Δ^9-desaturase activity in ethanol-fed rats. Similar results were generated by Rao et al. (1984) who fed rats 34% of calories as ethanol or dextrins and compared fatty acid synthase and SCD activities in liver tissue. Both enzyme activities were 50% in the alcohol-fed rats compared with those of the control rats.

When Nakamura et al. (1994) fed 40% of calories as ethanol to Yucatan barrow micropigs, hepatic microsomal Δ^5-desaturase activity was inhibited to 40% of the activity observed in control pigs, Δ^6-desaturase activity was inhibited to 28% of control, and Δ^9-desaurase activity increased numerically but not significantly. Because two of these desaturases were decreased in activity and the other one was not changed, it is probable that the inhibition is specific to the terminal desaturase and not to some supporting proteins and/or enzymes common to all three desaturases. The decrease in Δ^5- and Δ^6-desaturase activities was most noted by a decrease in the (n-3) series metabolites, but both (n-6) and (n-3) metabolites were decreased in concentration in the ethanol-fed pigs.

Angeletti and de Alaniz (1996) determined that ethanol addition to Hep G2 cells in culture inhibited logarithmic growth without affecting viability. Concentrations of cellular lipid classes did not change except for a slight increase in cholesteryl ester content. Evidently, ethanol inhibited fatty acid synthesis because 14:0 and 16:0 decreased in treated cells. Indirect evidence that the Δ^9-desaturase was inhibited by ethanol was noted when 16:0

accumulated as 18:0 and little was converted to 18:1(n-9) in cells treated with ethanol. Similarly, Δ^5-desaturase was inhibited because the conversion of 20:3(n-6) to 20:4(n-6) was inhibited in ethanol-fed cells. With ethanol supplementation, there was an increase in fatty acids, in the form of triacylglycerols, being exported from the cell. Hep G2 cells represent a faithful model of exposure of hepatocytes to ethanol, but the mechanisms of the noted changes are yet to be elucidated.

Cholesterol

Increased concentration of membrane cholesterol has been associated with decreased membrane fluidity, causing many to speculate about changed membrane protein function as a result. Garda and Brenner (1985) modified freshly prepared hepatic microsomes by incubating them with cholesterol and phospholipid liposomes. The cholesterol to phospholipid ratio in the microsomes ranged from 0.11 up to 0.8 in the modified microsomes. With increasing membrane cholesterol, decreases in membrane fluidity were measured by using fluorescent membrane probes. With decreased fluidity, NADH-cytochrome c reductase activity was decreased, but NADH-ferricyanide reductase activity was not affected. Delta-9-desaturase activity was increased with increased membrane cholesterol when 16:0, the assay substrate, was 2 μM but not when it was 66 μM. Delta-5 and Δ^6-desaturase activities were increased at either substrate concentration with increased cholesterol. These phenomena indicate that there is potentially enzyme regulation at the protein level which is controlled by the microenvironment of the enzyme in the membrane when Δ^5- and Δ^6-desaturases are concerned, but probably not for Δ^9-desaturase.

Contrary to *in vitro* studies, Garg et al., (1986) found that cholesterol-fed rats have enhanced Δ^9-desaturase activity and depressed Δ^5- and Δ^6-desaturase activities no matter if the rats are fed chow or chow with added sunflower oil or coconut oil. In fact, the largest changes in enzyme activity because of dietary cholesterol were in rats fed no supplemental fat. The changes in desaturase activities were supported by more 18:1(n-9) and less 20:4(n-6) in membranes of rats fed cholesterol. The authors speculate that the changes observed in desaturase activities are primarily because of changes in membrane fluidity brought about by dietary cholesterol. Osada et al. (1995) also demonstrated suppressed Δ^6-desaturase activity in rats fed cholesterol relative to rats fed control diets or oxidized cholesterol, whereas rats fed oxidized cholesterol had numerical increases in Δ^6-desaturase activity compared with that of control rats. Authors speculate that increased Δ^6-desaturase activity could lead to an increase in eicosanoid production when oxidized cholesterol is fed.

Leikin and Brenner (1987) also fed rats diets containing 1% cholesterol but added 0.5% cholate and studied hepatic microsomal fluidity, fatty acid composition, and desaturase activity. Membrane cholesterol and phosphatidylcholine concentrations both increased, but the cholesterol to phosphatidylcholine ratio increased. Saturated fatty acids and 20:4(n-6) decreased in the membranes while MUFA concentrations increased. A direct measure of fluidity indicated a decreased fluidity of the membrane as would be predicted by increased cholesterol content of the membrane. Microsomal Δ^9-desaturase activity increased as early as 48 hours after cholesterol feeding and Δ^5- and Δ^6-desaturase activities decreased. The desaturases maintained this change over the 21-day feeding period.

Landau et al. (1997) demonstrated that in rats fed corn oil, with or without added cholesterol, the cholesterol-fed animals had less of a decrease in liver SCD message and activity in a short time (48 hours). This study weakens the argument of Leikin and Brenner (1988) who speculated that the increase in SCD activity in cholesterol-fed animals was because the membranes lost fluidity because of increased cholesterol content. In the Landau study, the temporal order of events after cholesterol feeding was documented: 1. Increase in SCD message, 2. increase in SCD activity, and 3. increased cholesterol to phospholipid ratio. Because of an increase in SCD mRNA but no difference in transcription, it is speculated that cholesterol somehow increases message stability. The increased 18:1(n-9) production is consistent with an increase in cholesteryl esters because 18:1(n-9) is a preferred substrate for acyl-CoA:cholesterol acyl transferase. Part of the need for 18:1(n-9) during cholesterol feeding is to esterify the cholesterol and store it as cholesteryl oleate in the liver.

Hormones

General Introduction

A plethora of studies concerning hormone status or hormone administration on fatty acid metabolism have accumulated in the literature. Tissue from diabetic animals has less SCD activity than does that from non-diabetic animals, but, after insulin treatment of diabetic animals, the SCD activities are similar (Faas and Carter, 1980). Thyroxine injections given to normal rats causes an increased Δ^9-desaturase activity (Joshi and Aranda, 1979). Additional steroid hormones also cause increased SCD activity. Seventeen-β-estradiol enhances SCD activity in roosters (Lipiello et al., 1979), and testosterone enhances SCD activity in male or female rats (Marra and de Alaniz, 1989). Marra et al. (1988)

demonstrated that dexamethasone induces SCD in rat liver and in a hepatoma cell line via a cytosolic protein yet to be identified. Eleven-deoxycorticosterone, an example of a steroid hormone without an 11-OH group, also induces SCD activity via a cytosolic factor (Marra and de Alaniz, 1991). A more complete description of these and other hormone related studies follow.

Glucagon

Gomez-Dumm et al. (1975) speculated that many of the feeding effects observed on fatty acid desaturase activity were mediated via insulin and glucagon. To test the effects of glucagon on Δ^5-, Δ^6-, and Δ^9-desaturases, they compared fasted mice with refed mice given glucagon, dibutyryl adenosine 3'5'-cyclic monophosphate, or theophylline in different combinations. There were no differences by the imposed treatments on Δ^5- and Δ^9-desaturases except that fasted rats had very little enzyme activity compared with refed rats. Refeeding stimulated Δ^6-desaturase activity, measured with 18:2(n-6) or 18:3(n-3) as the substrate. The activity returned to the "fasted" level when glucagon or dibutyryl adenosine 3',5'-cyclic monophosphate was given with or without theophylline. With theophyllin alone, enzyme activity remained elevated. It was evident that glucagon was exerting an influence on Δ^6-desaturase activity via cyclic adenosine-3'5'-cyclic phosphate (cAMP). The authors point out that it is crucial for cells to have rigorous control over Δ^6-desaturase, the entry point of essential fatty acids into the desaturase/elongase system.

Adrenocorticotropic Hormone

Mandon et al. (1987) administered adrenocorticotropic hormone (ACTH) to rats and measured Δ^5- and Δ^6-desaturase activities in adrenal gland and liver to elucidate if the epinephrine-depressed desaturases results from concomitant ACTH release. Both *in vivo* and *in vitro* measures of Δ^5- and Δ^6-desaturase activities were depressed because of ACTH treatment. But, ACTH was shown to depress only Δ^5-desaturase activity in isolated hepatocytes where no glucocorticoid was present. One explanation could be that the "fast" response of ACTH is the result of a direct effect on enzyme activity, whereas the "slow" response could be via ACTH-stimulated glucocorticoid release.

Glucocorticoids

Glucocorticoids, including dexamethasone, cortisol, and corticosterone, and mineralocorticoids, including deoxycorticosterone and aldosterone as well as the estrogen β-estradiol and the androgen testosterone, enhanced Δ^9-desaturation of 18:0 in cultured rat hepatoma cells (Marra and de Alaniz, 1995). All hormones tested increased 18:0, or its metabolites, incorporation into phospholipids, except for deoxycorticosterone having little effect and testosterone having a slight inhibition. Unique patterns of labeling from labeled stearate within the lipid subclasses were noted for the various hormones, indicating an increased, but differential, incorporation of label. This experiment may help explain some of the observed physiological changes caused by using these hormones therapeutically or when they are not in balance such as in disease states.

Marra et al. (1986a) studied the dexamethasone inhibition of Δ^5- and Δ^6-desaturases in rat liver. The mode of inhibition was determined to be a soluble product found in the cytosolic fraction after membrane preparation. Most likely, the factor is a protein because it is trypsin labile. The cytosolic factor inhibits Δ^5- and Δ^6-desaturase activities *in vitro* when added to microsome preparations from non-treated rats. Marra et al. (1986b) previously had shown that synthesis of a glucocorticoid-induced inhibitor of Δ^5- and Δ^6-desaturases could be prevented by blocking protein synthesis with cycloheximide.

Growth Hormone

Growth hormone administration caused increased Δ^5-desaturase activity, no change in Δ^6-desaturase, and decreased Δ^9-desaturase activity in livers of male rats (Guéraud and Paris, 1997). Hypophysectomized rats had decreased Δ^5-desaturase, Δ^9-desaturase, and fatty acid elongase activities. Growth hormone given to males caused a "feminization" of the fatty acid composition of hepatic microsomal membranes.

Transgenic mice (MG101), expressing ovine growth-hormone when fed zinc, had increased expression of Δ^6-desaturase activity as measured *in vitro* and by comparing fatty acid composition of hepatic microsomes (Nakamura et al., 1996). In total fatty acids of adipose tissue, the products of the Δ^6-desaturase were enriched in treated mice compared with control mice, indicating increased expression of Δ^6-desaturase in adipose tissue of mice expressing excess growth hormone. Based on the fatty acid composition of phospholipid in adipose tissue, the treated mice have less Δ^6-desaturase activity than do control mice. The different mechanisms acting in hepatocytes and adipocytes are not known.

Mineralocorticoids

Marra et al. (1990) observed a decrease in hepatic Δ^6-desaturase activity after injecting the mineralocorticoids aldosterone and 11-deoxycoricosterone into rats. A 3-fold decrease in specific activity of Δ^6-desaturase as measured *in vitro* 24 hours after injection paralleled a dramatic (2- to 3-fold) decrease in 20:4(n-6) to 18:2(n-6) ratio in plasma as well as in liver microsomes. The physical character of the membrane as analyzed by fluorescence anisotropy also was changed, with the treated rats having higher anisotropy values (less fluidity).

Sex Hormones

Stearoyl CoA desaturase-1 mRNA is more than 4 times greater in female 7-week-old ICR mice than in littermate males (Lee et al., 1996). This message difference resulted in a 2-fold increase in 16:1(n-7) and a nearly 2-fold increase in 18:1(n-9) concentration in liver. The authors propose gender-specific hormonal differences as an explanation of their results. Medeiros et al. (1995) conducted correlation analysis on data from lean and obese women to identify insulin or estrogen as the primary regulator of desaturase activity. The obese women had increased insulin, but similar fasting glucose, compared with lean women. Estrogen decreased with age but was not affected by body mass index. Insulin was correlated positively with the ratio 20:4(n-6) to 18:3(n-6) in serum, an indicator of Δ^5-desaturase activity, and with the ratio 20:4(n-6) to 18:2(n-6) in serum, an indicator of overall (n-6) pathway activity. The only variable studied that correlated with estrogen was age, demonstrating that insulin is a stronger regulator of desaturase activity compared with estrogen.

Thorling and Hansen (1995) studied fatty acid composition of omental adipose tissue in male, female, castrated, and castrated-estrogen-treated rats. Mature female rats had more 18:1(n-9) in omental adipose than did male rats. Castrated males were intermediate to intact males and females but had nearly equal concentrations of 18:1(n-9) to females when given injections of estrogen. According to this study, it can be summarized that androgens and estrogens affect the accumulation of 18:1(n-9) in adipose tissue, possibly by decreasing and increasing SCD activity, respectively.

Triiodothyronine

van Doormaal et al. (1986) assayed fatty acid composition of blood lipid compartments in hypothyroid men in an attempt to understand Δ^6-desaturase activity in relation to thyroid status. Triiodothyronine supplementation of athyreotic patients with later withdrawal caused increases in plasma cholesterol, phospholipid, and triacylglycerol concentrations. The relative amount of 18:2(n-6) increased in all blood lipid compartments tested, whereas amount of 20:3(n-6) decreased. In plasma, all other (n-6) fatty acids, 22:6(n-3), 20:3(n-9), 16:0, 18:0 and total saturated fatty acids decreased, whereas MUFAs increased in the erythrocytes. The amount of 20:3(n-9) rose in erythrocytes, and the 20:3(n-9) to 20:4(n-6) ratio rose in polymorphonuclear leukocytes. This study indicates the crucial control of thyroid hormones over lipid metabolism, particularly Δ^6-desaturase, leading to potential disturbances in eicosanoid synthesis.

Ves Losada and Peluffo (1989) injected male rats with saturating doses of L-triiodothyronine (1000 μg T_3 per 100 g body weight) daily for 5 days. Hepatic microsomes were assayed for Δ^9-desaturase activity at eight time points throughout the period.

Desaturase activity was doubled in treated animals and was decreased to control activity when cycloheximide was injected, indicating a high rate of turnover of the enzyme. Surprisingly, hepatic membrane fatty acid composition of treated and control rats did not differ at the end of 5 days. In a separate publication, Ves Losada and Peluffo (1993) reported the changes in Δ^5- and Δ^6-desaturase response. There was about a 25% reduction in both desaturases when 1000 μg T_3 per 100g body weight was injected daily for 5 days. When half the dose was administered, only Δ^6-desaturase activity was decreased. There were no changes in desaturase activity before the 5^{th} day of the study independent of the dose. Changes in microsomal membrane fatty acid composition did not correspond to the changes observed when *in vitro* desaturase enzyme activity was measured, indicating that other mechanisms are acting to modify fatty acid composition of the membranes.

Insulin

One of the first studies to relate insulin to SCD was that of Prasad and Joshi (1979). They noted a 3.7-fold decrease in Δ^9-desaturase in liver of rats with stroptozotocin-induced diabetes. While insulin administration resulted in a 7-fold increase in Δ^9-desaturase activity in diabetic rats, fructose resulted in a 20-fold increase and glucose had no effect in diabetic rats. Because of the strong fructose effect, the authors speculated that insulin did not act alone to stimulate Δ^9-desaturase, but some intermediates of carbohydrate metabolism increase the activity of the terminal desaturase. This paper was also one of the first to demonstrate that carbohydrate metabolism had a significant effect on Δ^9-desaturase activity.

A study by Kawashima et al. (1985) compared the role of SCD on fatty acid composition of the microsomal membrane phospholipids. Rats had differing relative SCD activity in liver microsomes depending on how they were treated (starved < diabetic < control < fed clofibric acid < diabetic given insulin < refed after starvation). It was noted that, despite the divergent concentrations of 18:1(n-9) and 18:2(n-6) in the C-2 position of phosphatidylcholine, the sum of the two acids in the C-2 position was constant, indicating a direct competition for that position. There was a linear and direct association between the free 18:1(n-9) concentration of the microsomes and the 18:1(n-9) concentration in the C-2 position of phospholipids. The rats fed clofibric acid, however, had an increased amount of 18:1(n-9) at C-2 relative to the free 18:1(n-9) available on the microsome, indicating a preference shown by the acyltransferases required for phospholipid synthesis.

Streptozotocin, along with various concentrations of four different streptozotocin antagonists, was administered to rats to generate divergent concentrations of circulating insulin, triiodothyronine, and thyroxine (Nishida et al., 1988). When Δ^9-desaturase activity was plotted versus concentrations of all three hormones, sigmoidal curves with a positive slope were the result. Insulin gave a sigmoidal curve that had a more gradual slope, indicating that it regulates Δ^9-desaturase activity over a broader concentration range, whereas thyroid hormones acted more drastically to regulate Δ^9-desaturase over a much narrower concentration range.

Boustani et al. (1989) dosed diabetic and normal control humans with deuterium labeled 20:3(n-6) as a method to measure insulin-dependent Δ^5-desaturase activity. Deuterium labeled 20:4(n-6) appeared in plasma phospholipids about 12 hours after dosing and maximized between 48 and 72 hours after dosing. Labeled 20:4(n-6) was at the

detection limit in plasma triacylglycerols and not detectable in platelets. Arachidonic acid appearance in plasma phospholipids was about 50% in diabetic patients compared with controls. Insulin administered to the diabetic patients elevated 20:4(n-6) in plasma to the same concentration as in control patients, indicating the increased Δ^5-desaturase activity occurred via insulin.

Mimouni et al. (1992) studied the effects of insulin on Δ^9-desaturase by using Bio-Breeding rats that spontaneously develop diabetes. Hepatic microsomes were prepared from control rats and diabetic rats 3, 17, or 48 hours after their last insulin injection. *In vitro* Δ^9-desaturase activity assays indicated that the diabetic rats had 92, 64, and 50 percent of the Δ^9-desaturase activity at 3, 17, and 48 hours, respectivley, after their last insulin injection compared with that of control rats. The changes observed *in vitro* were not correlated with the fatty acid composition of liver membranes, but this result was not surprising considering the short time period involved.

Dosage and pattern of insulin administration was studied in the spontaneously diabetic Bio-Breeding rat as related to desaturase activities (Mimouni and Poisson, 1992). Delta-9-, Δ^6-, and Δ^5-desaturase activities were similar to those of the control rats when insulin was administered at the proper dosage of 10 I.U. twice a day. When the rats were held in a state of hyperglycemia by administering 1-half the dosage of insulin, Δ^9-, Δ^6-, and Δ^5-desaturase activities decreased to 25%, 42%, and 49% of control values, respectively. One dose of 10 I.U. insulin 17 hours before collecting microsomes (normoglycemia) resulted in all three desaturases being at about 65% of control values. Again, microsomal fatty acid compositions did not reflect all the changes in desaturase activity because of the short time

between changes in insulin status and sample collection and because of the multifactorial nature of fatty acid composition of animals.

Over a longer period of time, Shin et al. (1995) showed that insulin treatment of streptozotocin-induced diabetic rats restored the fatty acid composition of microsomal membranes. The disrupted fatty acid composition of membranes as a result of decreased Δ^6-desaturase activity associated with hypoinsulinemia was restored faster in the microsomal membrane than in the erythrocyte membranes upon insulin administration. The restoration of membrane fatty acid composition occurred because of insulin-induced Δ^6-desaturase activity, which was 68% and 119% of control rats before and after insulin therapy. This study also indicates that fatty acid composition of erythrocytes is not a good indicator of rapid changes in fatty acid metabolism.

Waters and Ntambi (1994) demonstrated that both insulin and fructose stimulate SCD1 gene expression in liver of diabetic mice. Fructose feeding to fasted diabetic mice caused a 23-fold increase in SCD1 mRNA in a 24-hour period whereas insulin caused a 22-fold increase during the same time period. Together, they resulted in a 45-fold increase in SCD1 mRNA concentration and a 10-fold increase in transcription 24 hours after administration. This experiment shows that posttranscriptional events occur when the mice are fed fructose that helps maintain SCD1 mRNA concentrations. One possible way the authors speculate that fructose could be acting is when insulin concentrations are decreased glucokinase is depressed but fructokinase and triosekinase are unaffected. Therefore, fructose itself or one of its metabolites could be acting to enhance SCD1 transcription. The up-regulation observed with insulin could be counteracted with cAMP derivatives, indicating that expression of SCD1 during fasting is inhibited by glucagon-stimulated cAMP.

Cycloheximide use also demonstrates the need for protein synthesis before the insulin effect can be observed.

Dang et al. (1989) fed normal and streptozotocin-injected diabetic rats different fatty acids to delineate changes in Δ^5-desaturase activity. As it has been noted, saturated fat feeding will increase the activity of Δ^9-desaturase relative to feeding unsaturated fatty acids. *In vitro* Δ^5-desaturase activity decreased, however, with feeding of saturated diets, did not change with unsaturated fat feeding, and decreased with fish oil feeding. The diabetic rats had an inherently depressed Δ^5-desaturase activity, which was not suppressed further by saturated fat or fish oil feeding. Feeding unsaturated fats enriched in (n-6) fatty acids did help restore activity but not to the same activity of control rats. Insulin dosing has been shown to correct Δ^6- and Δ^9-desaturase activities in diabetic animals, but it did not correct Δ^5-desaturase activity on the basis of *in vitro* enzyme assays or fatty acid composition of hepatic microsomes. An interesting observation was increased 22:6(n-3) in hepatic microsomes of diabetic rats fed fish oil, whereas 20:5(n-3) and 22:5(n-3) decreased despite being concentrated in the diet. The authors speculate that increased 22:6(n-3) and decreased 20:5(n-3) and 22:5(n-3) indicates a possible up-regulation of Δ^4-desaturase activity in livers of fish oil-fed diabetic rats.

The relationship between healthy insulin balance and fatty acid composition of membranes has been recognized increasingly as important (Storlien et al., 1997). The endogenous desaturase systems are very important in converting the average Western diet for humans that contains 80 double bonds per 100 fatty acids to skeletal muscle fatty acids that contain 180 double bond per 100 fatty acids. An index of Δ^5-desaturase activity, as measured

by the ratio of 20:4(n-6) to 20:3(n-6), has shown a correlation between decreased Δ^5-desaturase activity and insulin resistance (Borkman et al, 1993). When there is limited Δ^5-desaturase activity and there is an increase in dietary (n-6) fatty acids in an attempt to eat a "heart-healthy" diet, the membrane fatty acids can change dramatically. Under these conditions, competition for desaturation of the dietary fatty acid goes to the (n-6) acids that primarily end in 20:4(n-6) in the desaturation/elongation pathway, whereas the (n-3) fatty acids would have proceeded to pentaenoic and hexaenoic acids.

Differentiation

3T3-L1 Differentiation

The differentiation process of 3T3-L1 mouse preadipocytes maturing into lipid-laden adipocytes has been one of the pillars for studying both the enzymology and molecular biology associated with lipid metabolism. Kasuri and Joshi (1982) studied hormonal regulation of SCD and lipogenesis during the differentiation of 3T3-L1 preadipocytes into adipocytes. During differentiation, the rate of lipid desaturation increased 5.9-fold and the rate of fatty acid synthesis increased 255-fold. Stearoyl-CoA desaturase, fatty acid synthetase, and malic enzyme activities also could be induced in fully differentiated cells by 100-, 63-, and 50-fold, respectively, by providing insulin. Despite the dependence of SCD on NADH-cytochrome b_5 reductase and cytochrome b_5 itself, the most regulated and limiting factor during and after differentiation proved to be the terminal desaturase. Biotin deficiency definitely slowed the synthesis of saturated fatty acids, but decreased biotin or saturated fatty acids did not decrease the ability of cells to desaturate exogenously attained fatty acids. While insulin-stimulated differentiation is slower than stimulation with dexamethasone, 1-

methyl-3-isobutylxanthine, and insulin, addition of insulin antibodies efficiently inhibits differentiation.

The molecular biological approach of studying SCD began in 1986 when Thiede et al. isolated a cDNA from rat that coded for SCD. In 1988, Ntambi et al. isolated and characterized the cDNA and gene responsible for production of SCD from 3T3-L1 cells. The open reading frame codes for a protein with 92% homology to the cloned rat liver SCD. The message for SCD contains an unusually long 3' untranslated region (UTR)(3.5 kb) and a large 5' UTR. While the gene is expressed constitutively in murine adipose tissue, it is increased in the liver when mice are starved and refed a high-carbohydrate diet. The isolated gene is 15 kb and includes six exons and five introns. The promoter region contained homologous sequences to putative fat-specific transcriptional element (FSE2), cAMP, and glucocorticoid regulatory elements. To confirm differentiation-induced expression, the promoter region was fused to the CAT gene and CAT expression was measured in 3T3-L1 cells only after differentiation protocols were initiated.

Kaestner et al. (1989) identified two different genes encoding SCD from mouse 3T3-L1 cells. They have been designated SCD1, which is exclusively expressed in the liver, and SCD2, which is expressed exclusively in spleen, heart, and brain. Adipose, lung, and kidney tissues express both forms to varying extents. Stearoyl-CoA desaturase-1 and SCD2 have greater than 87% sequence identity. The genes for the two desaturases have similar intron/exon junctions and similar regulatory regions in the promoter. Whereas SCD2 lacks the TATA box in the 5' region compared to SCD1, it does have a 140-bp sequence in the promoter that shares 77% identity with the SCD1 promoter. Stearoyl-CoA desaturase-2 has two CCAAT boxes, a GC-rich region similar to proven Sp1 transcription factor binding

regions, consensus sequence for the glucocorticoid regulatory element, and CCAAT box/enhancer binding protein sequence. The promoter regions were immediately noted as being rich in known and putative regulatory sequences, and this cell line continues today to be a useful tool for examination of gene regulation related to lipid metabolism. In 1995, Ntambi summarized the research on SCD1 and SCD2 expression during differentiation of adipocytes and as regulated by dietary factors.

The counter regulation of SCD1 and acyl-CoA synthetase (ACS) by insulin and tumor necrosis factor-α (TNF-α) in 3T3-L1 was studied by Weiner et al. (1991). Insulin stimulates a 4-fold increase in rate of transcription of these two lipogenic genes, but TNF-α causes greater than 75% decrease in mRNA expression for both ACS and SCD1. Other lipogenic genes such as malic enzyme and lipoprotein lipase are not regulated transcriptionally by either of these two factors.

Casimir and Ntambi (1996) evaluated the effects of cAMP on SCD1 expression in 3T3-L1 preadipocytes as they differentiated into lipid-laden adipocytes. The induction protocol for differentiating preadipocytes into mature adipocytes includes a cocktail of methylisobutylxanthine, dexamethasone, and insulin. It was determined that methylisobutylxanthine was the only compound responsible for SCD1 induction, presumably via its ability to generate cAMP by inhibition of cAMP phosphodiesterase. Cell-permeable cAMP analogs resulted in similar results. By conducting deletion analysis of chimeric SCD1-promoter/reporter gene constructs, it was shown that the cAMP response requires the –253 to +30 region and that constructs from –253 to –76 and –76 to +30 both respond to cAMP, indicating multiple response elements. Both regions contain an AP-2 response element. The rapid (minutes) response observed with genes that are induced via the

cAMP/AP-2 mechanism does not occur with the cAMP-induced expression of SCD1. Therefore, the obvious induction of SCD1 by cAMP is not direct.

Sessler et al. (1996) provided 20:4(n-6) to 3T3-L1 cells in culture and observed decreased SCD1 activity and mRNA concentrations without changing the rate of transcription. The half-life of SCD message in the presence of 20:4(n-6) dropped to 8.5 hours from 25.1 hours in control cells. Blocking the conversion of 20:4(n-6) to eicosanoids did not change the result, indicating that 20:4(n-6) is the responsible compound. As had been demonstrated in the past, saturated fatty acids or MUFAs did not change SCD1 mRNA or enzyme concentrations, but 18:2(n-6) and 18:3(n-3) behaved similarly to 20:4(n-6). The 60% decrease in enzyme activity caused by feeding cells 20:4(n-6) could be accounted for by the 80% decrease in SCD1 mRNA concentration because of loss of message stability and no changes in transcription as is observed in the liver. The authors speculate that the long 3' tail of SCD1 message may play a role in controlling the half-life of the mRNA.

Development

One of the first papers describing SCD activity during phases of development was by Wilson et al. (1976). They studied the expression of SCD in newly hatched chicks through 6 days of age. At hatch, hepatic microsomes were lacking in SCD activity (0.05 nmol/min•mg), but, by 6 days of age, the activity had increased 100-fold (4-5 mnol/min•mg). Supporting enzymes, proteins, and cofactors were assayed and did not change significantly through this time. When partially purified desaturase was added to microsomes from newly hatched chicks, a fully functional desaturase system was observed,

indicating that induction of the terminal desaturase occurs during the first days of a chick's life.

In the mammal, the placenta provides a barrier to protect the developing fetus from the mothers immune system but at the same time prevents some nutrients from entering into the fetal blood supply. Descomps and Rodriguez (1995) studied the disparity of 20:4(n-6) and 22:6(n-3) being 2-fold greater in fetal plasma compared with that in maternal plasma, whereas just the opposite is true for their precursors 18:2(n-6) and 18:3(n-3). The disparity between maternal and fetal blood fatty acid composition raises questions about which fatty acids should be included in prepared infant formulas. Supplementation of formulas for premature infants with 4.95% of total energy as 18:2(n-6) and 0.77% as 18:3(n-3) resulted in blood fatty acid profiles that were more similar to infants fed breast milk than that in infants fed typical formula. Based on *in vitro* assays of Δ^5- and Δ^6-desaturase activities, it is crucial to maintain the 6.4 to 1 molar ratio of 18:2(n-6) to 18:3(n-3) to prevent substrate inhibition. The authors concluded that human fetal liver has the ability to desaturate fatty acids and that 18:2(n-6) and 18:3(n-3) supplementation, when provided in the correct ratio, should fulfill the infants essential fatty acid requirement.

Genes critical to myelin formation in the developing mouse brain were studied by Garbay et al. (1997). Myelin is 70-85% lipid and quadruples in mass from 2 weeks of age to 6 months of age, whereas the brain weight only increases by 50% during this time. Oleic acid, the product of SCD2, comprises up to 40% of myelin fatty acids. Maximal myelination occurs at day 20 after birth in rats and mice. Ceramide galactosyltransferase, a critical enzyme in myelin formation, had maximal expression at about day 18 in this study. Messenger RNA concentrations for acetyl-CoA carboxylase and fatty acid synthase are

maximal 5 days after birth and decline to 20% of maximal concentration at day 20. Expression of SCD2 is different from that of other genes during active myelination because it remains near maximal from birth to about day 10 and then gradually declines to about 60% of maximal at day 40. This study confirms that regulation must occur at the transcriptional level for enzymes crucial to myelination.

de la Presa-Owens et al. (1998) compared tissue fatty acid composition and desaturase activities after addition of 20:4(n-6) or 22:6(n-3) in dietary triacylglycerols in formula fed to pigs. The underlying concern is the use of plant lipids in infant formulas that contain the essential fatty acids 18:2(n-6) and 18:3(n-3) but little 20:4(n-6) and 22:6(n-3) as found in human breast milk. During rapid post-natal development, there is concern about the ability of infants to desaturate and elongate dietary fatty acids for use in critical growth such as that of neural and organ tissues. The authors found that the dietary fatty acids were enriched in plasma and tissue depots except for brain, which remained resistant to change despite dietary fatty acid supplementation.

Peroxisome Proliferators

Kawashima and Kozuka (1982) noted 4-fold increases in SCD activity in rat liver after feeding clofibric acid. The increase in activity was determined to be because of the terminal desaturase and not the activity of NADH-cytochrome b_5 reductase or the availability of cytochrome b_5. Similar inductions of enzyme activity were noted in clofibrate-fed rats that had been afflicted with hormone anomalies or given hormone injections, creating or mimicking diabetes, hyperthyroidism, or hypothyroidism. Therefore, the mechanism of clofibrate action is probably not via changes in insulin or thyroid status and probably is not

hormonally associated. Changes in SCD activity were reflected by increased concentrations of 18:1(n-9) in livers and even more markedly in the isolated hepatic microsomes of treated rats.

In a study conducted later, Kawashima et al. (1986) fed two different peroxisome proliferators, clofibric acid or tiadenol, to rats, mice, and guinea pigs to study changes in microsomal lipid composition and the enzyme systems responsible for any observed changes. Rats and mice responded similarly in that 18:1(n-9), via SCD, increased in phosphatidylcholine of hepatic microsomes, whereas guinea pigs did not respond. Supporting enzymes such as palmitoyl-CoA elongase, 1-acylglycerophosphate acyltransferase, and 1-acylglycerophosphocholine acyltransferase also increased after feeding peroxisome proliferators, indicating orchestrated changes in lipid metabolism, not just modulation of SCD, by peroxisome proliferators.

Kawashima et al. (1989) fed clofibric acid, tiadenol, and perfluoro-octanoic acid to male and female mice. All three compounds increased Δ^9-desaturase activity in liver except perfluoro-octanoic acid did not cause a response in female mice, making perfluorinated carboxylic acids the only known peroxisome proliferator that is affected by gonadal hormones. The changes were measured by *in vitro* assays and confirmed by comparing phospholipid fatty acid composition of microsomal membranes. Fatty acid composition data agreed well with enzyme assay data collected by *in vitro* assays.

Diczfalusy et at. (1995) imposed clofibrate treatments on mice and quantified both SCD1 and SCD2 mRNA in several tissues along with SCD1 activity in the liver. As expected, SCD1 was expressed in liver and brown adipose tissue, whereas SCD2 was expressed in brown adipose tissue and brain. Diurnal variation of hepatic SCD1 existed, and

expression was found to be greatest during the feeding period. Clofibrate increased hepatic mRNA and *in vitro* activity of SCD1 by around 2-fold and 1.5-fold, respectively. The tissue with the highest concentration of message for both SCD1 and SCD2 was brown adipose tissue, which was down-regulated by feeding a fat-free diet. This observation has no current explanation and warrants further investigation

It has been noted that clofibric acid increases selectively the proportion of 18:1(n-9) in the C-2 position of phospholipids, but 18:1(n-9) is usually distributed equally between C-1 and C-2 positions. The two primary enzymes required to accommodate this change are SCD and 1-acylglycerophosphocholine acyltransferase. Three peroxisome proliferators (4-chlorophenoxyisobutyric acid [clofibric acid], 2,2'-(decamethylenediothio)-di-ethanol [tiadenol], and perfluoro-octanoic acid) at various dosages were fed to rats to induce SCD and 1-acylglycerophosphocholine acyltransferase. This experiment was an attempt to understand which of the two enzymes was most responsible for the large increase in 18:1(n-9) in the C-2 position of phosphatidylcholine (Mizuguchi et al., 1996). Multiple regression analysis revealed correlations between the concentration of both enzymes and 18:1(n-9) enrichment at the C-2 position of phosphatidylcholine, but SCD was by far more strongly correlated and therefore most responsible.

The increase of SCD1 mRNA after feeding peroxisome proliferators to mice was shown to be at the level of transcription by using nuclear run-on assays in nuclei from mice fed clofibrate (Miller and Ntambi, 1996). Deletion analysis of the SCD1/CAT construct identified AGGTCA at position –664 in the promoter for SCD1 as a cis-linked response element for the PPAR. These data put SCD1 expression in a class of its own because it is a lipogenic gene that is down-regulated by PUFAs, but peroxisome proliferators also induce its

expression. The blood lipid-lowering effects of peroxisome proliferators acts via increasing hepatic clearance of triacylglycerols (Reddy and Lalwai, 1983). This results in an increase in enzymes required for β-oxidation and phospholipid synthesis and a decrease in enzymes associated with fatty acid synthesis. In contrast, Δ^5-, Δ^6-, Δ^9-desaturase and elongase activities are increased by peroxisome proliferators. It is thought that the desaturases are increased because of the great need for membrane phospholipid synthesis and the great increase in β-oxidation; therefore, the endogenous desaturase system must be up-regulated to help support phospholipid synthesis.

Perfluoro-decanoic acid is another known peroxisome proliferator that produces confusing results with respect to SCD. Yamamoto and Kawashima (1997) fed rats 0.005% perfluoro-decanoic acid (w/w) for 7 days. In this scenario, perfluoro-decanoic acid acted as a typical peroxisome proliferator because it up-regulated SCD. *In vitro,* however, perfluoro-decanoic acid acts as an inhibitor of the terminal desaturase by interfering with the electron transport system required to support desaturase activity. The doses given to the rats were probably enough to activate the transcriptional machinery of the cell but not enough to inhibit the terminal desaturase *in vivo*.

Thiazolidinediones (TZDs), developed for the treatment of non-insulin-dependent diabetes, activate PPAR-γ, but result in down-regulation of SCD1 expression in fully differentiated 3T3-L1 cells (Kurebayashi et al., 1997). Stearoyl-CoA desaturase-1 mRNA was identified by using differential display techniques as being down-regulated with TZD treatment. The fatty acid composition of the TZD-treated cells shows a significant increase in saturated fatty acids and a decrease in MUFAs. Kliewer et al. (1997) has documented an

increase in insulin secretion from rat pancreas perfused with saturated fatty acids as a possible explanation of the mode of action for TZDs.

Inhibitors

Inhibitors of fatty acid desaturases in animals could be important in treating disease, producing food products of specific fatty acid composition, or learning about metabolic pathways *in vivo*. It has been demonstrated that dietary cyclopropene fatty acids, such as sterculate, cause a decreased in MUFAs and an increase in the corresponding saturated acids in tissue lipids. Pande and Mead (1970) published an experiment where sterculate seemed to inhibit desaturases *in vivo* because of some undetermined mechanism, but in *in vitro* experiments the inhibition was simply a detergent effect of sterculate and not competitive inhibition or interaction of desaturase sulfhydryl groups with the cyclopropene ring.

Raju and Reiser (1972) confirmed that sterculate was a specific and potent inhibitor of SCD and was not acting as a nonspecific detergent. To arrive at this conclusion, sterculate was shown to inhibit the SCD reaction much more potently than did 18:1(n-9) and its potency was not decreased when more microsomal protein was added to the reaction, which would have diluted out a detergent effect. The mode of inhibition was suspected to be via binding of sterculate to sulfhydryl groups of the protein, but the sequence of SCD was not known at the time to confirm this as a possibility. Sterculic acid also has been shown by Zoeller and Wood (1985) to inhibit SCD in the Morris hepatoma 7288C cell line. Sterculic acid at 20-30 μM inhibited production of monoenoic fatty acids via *de novo* synthesis by 90% and decreased 18:1(n-9) content of the cell by 50%.

Sterculic acid was used to inhibit implanted tumor growth in rats (Khoo et al., 1991). When administered subcutaneously for 4 weeks prior to and 4 weeks after tumor implantation, the tumor volume was decreased by more than 3-fold. Oleic acid content of liver, plasma, erythrocytes, and tumor tissue was decreased in the treated rats compared with the control rats. It is highly likely that the changes observed in tumor fatty acid composition were the reasons for the decrease in tumor growth-rate, whereas the rate of newly implanted tumors taking hold and becoming established was not changed with sterculic acid treatment. On the basis of the increased ratio of 18:2(n-6) to 20:4(n-6), it is suggested that sterculic acid is also a Δ^6-desaturase inhibitor. Brain fatty acid composition was not affected however. The mechanism of sterculic acid inhibiting tumor growth has not been identified, but the most probable mode is speculated to be the decreased fluidity of cell membranes, which could confer differences in surface protein activity of crucial receptors.

It was noted that sesame seed oil inhibited mycelial 20:3(n-6) conversion to 20:4(n-6) (Shimizu et al., 1991). Fractions of sesame seed oil, separated by high-performance liquid chromtography (HPLC), were assessed for their ability to inhibit *in vitro* Δ^5-desaturase reactions. The active compound, as identified by mass spectrometry, ^{1}H NMR, optical rotation, and melting point, was (+)-sesamin. When kinetic inhibition studies were conducted on fungal and mammalian (rat) Δ^5-desaturases, (+)-sesamin was found to be a potent and specific non-competitive inhibitor of Δ^5-desaturase. This was the first identified natural inhibitor of the Δ^5-desaturase enzyme.

Eritadenine, a hypocholesterolemic factor of the mushroom *Lentinus edodes,* is another natural fatty acid desaturase inhibitor. It inhibits Δ^6-desaturase activity in rat liver

microsomes. The phosphatidylcholine to phosphatidylethanolamine ratio in eritadenine-fed rats decreases in a dose-dependent manner (Sugiyama et al., 1997). In vitro manipulation of the phospholipid composition of liver microsomes by phospholipase C indicates that Δ^6-desaturase activity can be modified by phospholipid composition (Leikin and Shinitzy, 1995).

Obukowicz et al. (1998) identified and examined a compound named CP-24879 as a potential Δ^5- and Δ^6-desaturase inhibitor. The hypothesis was that inhibition of Δ^5- and Δ^6-desaturases could result in an anti-inflammatory response. When mouse mastocytoma cells ABMC-7 were cultured with the compound, there was a dose-dependent decrease in (a) desaturase activity, (b) 20:4(n-6) production, and (c) leukotriene C_4 production. When maximally tolerated doses of CP-24879 were given to mice, the combined Δ^5- and Δ^6-desaturase activities were inhibited 80% and 20:4(n-6) was depleted by 50% leading to a predicted decrease in ensuing eicosanoid production. Chronic use of a drug such as this would probably not work well because of the disease states known to exist when defective Δ^6-destuarase activities are present, such as diabetic neuropathy and chronic inflammation disease, but short-term drug therapy may prove useful.

Membrane Fluidity

Fatty acid composition of liver microsomes were modified by feeding rats choline-free diets for up to 21 days (Leikin and Brenner, 1992). As phosphatidylcholine concentration decreased in the membranes, phosphatidylserine and cholesterol increased, resulting in a decrease in membrane fluidity as measured by fluorescence anisotropy. Along with the decrease in fluidity was a decrease in Δ^5-desaturase activity and a concomitant

decrease in 20:4(n-6) content of the membranes. This study along with the study of Garda and Brenner (1985), where cholesterol added to microsomal membranes *in vitro* increased Δ^5-desaturase activity, demonstrate that trying to correlate desaturase activity directly with membrane fluidity may not be accurate. A more detailed look at phospholipid ratios, cholesterol content, and fatty acid composition of the membrane make predicting desaturase activities difficult.

When considering the effects of dietary fatty acids, it is useful to know the minimal response time of the criteria being measured in trying to define possible mechanisms. Dietary fatty acids affected rat hepatic membrane composition and desaturase activities as quickly as 6 days after initiating a diet with 10% fat from coconut oil, olive oil, or sunflower oil (Girón and Suárez, 1996). Delta-9-desaturase activity was significantly different in all three groups at day 6 with sunflower oil-fed rats having the least Δ^9-desaturase activity and olive oil-fed rats having the greatest. Delta-6-desaturase was significantly increased at day 6 in the olive oil- and sunflower oil-fed animals relative to the coconut oil fed animals.

Foucher et al. (1997) compared membrane fluidity, desaturase activities, and membrane fatty acid composition in normal and spontaneously hypertensive rats (SHR). Based on electron spin resonance of 10-nitroxide 18:0, the microsomal membranes of SHR were more fluid than those from control rats. When fatty acid composition and desaturase activity assays were compared, the decrease in Δ^6-desaturase of the SHR paralleled the decrease in 20:4(n-6). The decrease in Δ^9-desaturase observed *in vitro* for the SHR, however, was not consistent with a significant increase in 18:1(n-9) in the SHR. The authors suggest that the increase in membrane fluidity of SHR may result in a viscotropic regulation of fatty acid desaturases and explain the decrease in Δ^6- and Δ^9-desaturase activities. They

also suggest that the PUFA biosynthesis impairment is related to change in the microenvironment of the desaturases.

Environmental Temperatures

Kasai and Nozawa (1980) tested the theory that has been presented for Δ^9-desaturase activity regulation based on changes in membrane fluidity. They summarized that decreasing the temperature of starved tetrahymena (no detectable Δ^9-desaturase) quickly induces the desaturase capabilities of these organisms as measured by *in vivo* conversion and *in vitro* assays.

Brain lipids in hibernating hamsters are enriched in MUFAs (Goldman, 1975). Goldman (1978) identified that MUFAs in brain tissue must come from exogenous sources, as SCD was not increased in brain tissue of hibernating hamsters. Liver tissue, however, had an 8-fold increase in desaturase activity when hibernating versus warm-adapted hamsters were compared. The liver could supply the brain with MUFAs via lipoprotein transport.

Gonzalez et al. (1983) studied fatty acid desaturase activity in rats during the transition from warm adaptation (32° C) to cold adaptation (15° C). In female rats, Δ^9-desaturase and fatty acid synthetase were decreased in liver microsomes 24 hours after cold exposure, whereas Δ^5- and Δ^6-desaturases were increased. The changes in fatty acid desaturase activity were evident by the increase in 20:4(n-6) and the decrease in 18:1(n-9) in membrane phospholipids. Desaturase activities in male rats subjected to the same conditions did not change, indicating a possible link between cold adaptation and gender in rats. To study gender-specific hormone effects, Gonzalez et al. (1986) studied the effects of temperature change on hepatic Δ^6-desaturase in intact and ovariectomized female rats. The

intact rats had an increased Δ^6-desaturase system when switched to 15° C after having been acclimated to a 32° C environment. The increase was documented by *in vitro* enzyme assays and increases in microsomal 20:4(n-6). The ovariectomized rats treated to the same temperature protocol had increased membrane 20:4(n-6), but no change in desaturase activity was measured *in vitro*. Estradiol concentration in plasma of intact rats was higher in the warm acclimated rats compared with when the rats were switched to the cold environment. To test if ovaric sex hormones were depressing Δ^6-desaturase, 17-β estradiol was injected into intact and ovariectomized female rats maintained at 15° C and 30° C. In all cases, Δ^6-desaturase decreased, indicating that estradiol is a depressor of Δ^6-desaturase activity.

Ves Losada and Peluffo (1987a) studied the effects of a cold environment on hepatic microsomal Δ^6- and Δ^9-desaturase activities in male rats. There was no change in Δ^6-desaturase activity as measured *in vitro* or by analyzing microsomal membrane fatty acid composition. Unlike in fish, where Tiku et al. (1996) observed an up-regulation of Δ^9-desaturase in hepatocytes, there was a decrease in Δ^9-desaturase activity in mice maintained at 5° C compared to that in those kept at 24° C. When rats were pair-fed, it was determined that the down-regulation of Δ^9-desaturase was based on restricted energy available for lipogenesis and not a direct effect of the colder environmental temperature.

In a similar study, Ves Losada and Peluffo (1987b) measured Δ^5- and Δ^6-desaturase activities in liver of male rats before and after a decrease in environmental temperature. When shifted from an adapted temperature of 24° C to a 12° C environment, there was no difference in Δ^5- or Δ^6-desaturase activities unless the rats were first fed a hyperglycidic diet (carbohydrate enrichment in place of protein). The depression of Δ^5- and Δ^6-desaturases as a

result of the hyperglycidic diet was compensated for by the increase in activities of both enzymes when the environmental temperature was decreased to 12° C. Therefore, it can be said that cold evokes an increase in Δ^5- and Δ^6-desaturases in male rats only when these enzymes are first suppressed by dietary means.

Modes of temperature induction of unsaturated acid formation have been studied in many species. Guerzoni et al. (1997) have proposed a novel mechanism of MUFA formation in *Saccharomyces cerevisiae* under superoptimal thermal stress. They and other groups have demonstrated an increase in saturated fatty acids in yeast as the temperature is increased from 10° C to 37° C. However, their question was: why is there an increase in MUFAs as the temperature continues to increase into the superoptimal range (42° C)? Thermotolerant yeast have 3-fold lower thiobarbituric acid reactive substances (TBARs) than do common strains when grown at 42° C. Their conclusion was that in a thermotolerant strain of yeast, the use of oxygen for the desaturation reaction is a way to decrease the damaging oxidative products that increase in the membrane as the temperature approaches the superoptimal range.

Disease

Aging

Aging is a mysterious and long-studied process. One of the documented science-based processes related to aging is a dramatic drop in Δ^6-desaturase activity. Dietary 18:3(n-6) bypasses the need for Δ^6-desaturase activity in the (n-6) pathway and restores production of 20:3(n-6) and prostaglandin E1. Unfortunately, foods rich in 18:3(n-6) such as human milk, evening primrose oil, and borage oil are not common dietary components. The most likely problems to arise when prostaglandin E1 concentrations in tissue membranes drop are a

decrease in activated T-cells, increases in blood pressure, increased risk of malignancy, and a drop in cAMP concentration in various tissues (Horrobin, 1981), which are all conditions often correlated with aging.

Bordoni et al. (1988) correlated Δ^6-desaturase activity and microsomal membrane fatty acid composition by using aging rats. There were no differences between suckling and weanling rats, but from weaning until senescence there was a linear decrease in hepatic Δ^6-desaturase activity and a predicted decrease in the 20:4(n-6) to 18:2(n-6) weight ratio of microsomal membranes. Hrlia et al. (1990) studied the substrate-dependent kinetics of Δ^6-desaturase in aging rats. When 18:2(n-6) was used as a substrate, the K_m increased linearly with age and there was no change in V_{max} until 25 months of age. With 18:3(n-3) as a substrate, however, there was no change in K_m but V_{max} increased with aging.

The expression patterns of Δ^6-desaturase activity in mouse brain and liver during normal development differ greatly (Bourre et al., 1990). From day 2 before birth until 21 days after birth, the activity of brain Δ^6-desaturase activity declines about 10-fold and then maintains stable activity until 17 months of age when this study ended. Hepatic Δ^6-desaturase, however, increased from day 2 before birth until day 7 after birth, declined rapidly until day 13, and then declined gradually until 4 months when the slope of decline increased until 17 months. Overall, there was a 40% decrease in hepatic Δ^6-desaturase activity between 4 and 17 months. The activity of hepatic Δ^6-desaturase was 10-to 20-fold greater after weaning compared with values obtained at the end of the study.

Maniongui et al. (1993) compared the activities of Δ^5- and Δ^6-desaturases with aging in rat liver microsomes. Delta-6-desaturase activity with 18:3(n-3) as a substrate increased

from 1.5 to 3 months of age and then decreased linearly to 24 months of age only to return to the original value observed at 1.5 months. With 18:2(n-6) as a substrate, Δ^6-desaturase activity increased up to 6 months and then remained constant until 24 months of age. Delta-5 desaturase activity decreased from 1.5 months of age to 3 months of age and then increased until 24 months of age when it regained the same activity as it had at 1.5 months of age. The age-related changes in desaturase activity were reflected in the observed fatty acid composition of microsomal phospholipid fatty acids. Arachidonic acid increased in both red cell membranes and microsomal phospholipids throughout the aging study, whereas total (n-3) acids did not vary with aging.

Jimenez et al. (1993) were the first to document Δ^6-desaturase activity in heart microsomes prepared from rats. The age-related decrease observed by most investigators in hepatic Δ^6-desaturase also occurs in heart tissue but to a lesser extent. Despite measurable decreases in Δ^6-desaturase activity with aging in heart tissue, microsomal fatty acid composition did not change.

Essential fatty acid status has been implicated with many disease-related processes such as coronary heart disease (Wood et al., 1987), diabetes mellitus (Horrobin, 1993), and rheumatoid arthritis (Belch and Maple, 1992). To better understand the activity of Δ^6-desaturase activity related to aging in humans, Bolton-Smith et al. (1997) compared the fatty acid composition of adipose biopsies from over 4,000 men and women. When adjustments were made for dietary fats, body mass index, and smoking, the ratio of 18:2(n-6) to 18:3(n-6) increased with age in both men and women, indicating a decreased activity of Δ^6-desaturase

enzyme with aging. Increasing dietary 18:3(n-6) intake with age is suggested but not yet accepted as a strategy to overcome the age-associated decline in Δ^6-desaturase activity.

Aging as related to 18:2(n-6) modification and incorporation into lipids was studied in human skin fibroblasts at various passages ranging from 3 to 30 (Raederstorff et al., 1994). While total uptake of 18:2(n-6) into cells did not differ in passage three compared to passage 30, the composition of the metabolized 18:2(n-6) did change. The ratio of 20:4(n-6) to 18:2(n-6) which reflects the combined Δ^5- and Δ^6-desaturase capacities of the cells, and the 20:3(n-6) to 18:2(n-6) ratio as a measure of Δ^6-desaturase capacity decreased 66% and 58%, respectively, from passage three to passage 30. When the 20:4(n-6) to 20:3(n-6) ratio of the passaged cells was compared, the Δ^5-desaturase activity decreased 14% from passage three to 30. Thus, during *in vitro* aging, the rate limiting Δ^6-desaturase displays the greatest loss of activity compared with Δ^5-desaturase.

The kinetics of Δ^6-desaturase related to aging in rats was studied by Hrelia et al. (1990). There was little effect on kinetic parameters of Δ^6-desaturase when young rats were fed 18:3(n-6), but when old rats were fed 18:3(n-6) the V_{max} for both 18:2(n-6) and 18:3(n-3) were increased, and K_m values did not change. In fact, the V_{max} in old rats fed 18:3(n-6) was greater than that in young, pair-fed control rats. It seems probable that feeding small amounts of 18:3(n-6) containing evening primrose oil can restore Δ^6-desaturase activity normally lost during aging. Going one step further, presumably the increased availability of *de novo* synthesized pentaenoic and hexaenoic acids would maintain proper membrane fluidity during aging.

Biagi et al. (1991) also studied the effects of dietary 18:3(n-6) on hepatic microsomal Δ^6-desaturase activity in aged rats. Old rats were fed diets containing 50% of dietary lipid from evening primrose oil or from soybean oil, which provided about 6% 18:3(n-6) to those rats fed evening primrose oil. In the young rat, there was no change in Δ^6-desaturase activity as measured by using 18:2(n-6) or 18:3(n-3) as the assay substrate. In the old animals, however, 18:3(n-6) feeding enhanced Δ^6-desaturase activity 36% and 79% with 18:2(n-6) and 18:3(n-3), respectively, as substrates. Even with 18:3(n-6) feeding, the Δ^6-desaturase activity observed in old rats with 18:2(n-6) as the substrate was about 50% of the rate observed in young rats. When 18:3(n-3) was used as a substrate, however, the old rats fed 18:3(n-6) had more than 150% of Δ^6-desaturase activity compared with young rats.

Dinh et al. (1993) studied the effects of aging and 18:3(n-3) deficiency on Δ^6-desaturase activity in rats. As others have shown, Δ^6-desaturase activity decreases with age but the rate and pattern in which it decreases was demonstrated to be diet-dependent. Comparable with other studies, the Δ^6-desaturase rate was stable until about 6 months of age, decreased 30% from 6 to 12 months of age, and then remained stable until 24 months of age. The Δ^6-desaturase activity in the fatty acid-deficient rats did not decrease throughout the study. It was interesting to note that refeeding essential fatty acids to depleted rats resulted in decreased total liver PUFA in young rats but increased liver PUFA in older rats. Fish oil refeeding resulted in a more rapid and complete recovery than simply feeding 18:3(n-3) to the older rats, but either refeeding regime was acceptable for younger rats that possess maximal Δ^5- and Δ^6-desaturase systems.

Recovery of full Δ^6-desaturase activity after being suppressed by dietary fish oil was not as rapid or complete in old rats (24 months old) compared with recovery of young rats (9 months old) (Dihn et al., 1995). Young rats had regained enzyme activity similar to that of control rats after 3 days of feeding a low (n-3) diet, with a slight additional increase at 7 days. The old rats, however, regained only about 65% of control activity after being fed a low (n-3) diet for 7 days. The liver phospholipid fatty acid composition had not fully recovered to its original composition 7 days after switching back to a low (n-3) diet but had changed considerably because of increased Δ^6-desaturase activity. The brain phospholipids, however, remained resistant to change during this time period.

Cancer

Hepatic tumors have been linked to long-term peroxisome proliferator administration in rats and mice (Reddy and Lalwai, 1983). The association of enhanced SCD activity with peroxisome proliferators and increased 18:1(n-9) synthesis in some types of tumors, which increases membrane fluidity, have led to speculation about the possible carcinogenic nature of peroxisome proliferators. A contradiction to this theme was examined by Zoeller et al. (1984). They analyzed the SCD system in the Morris hepatoma cell line 7288C in culture and in 7288CTC maintained in host rats. These cells had extremely decreased SCD activity compared with normal liver tissue (4% and 8% respectively). The reason for the observed decrease in terminal desaturase activity may be because of decreased concentrations of cytochrome b_5 (2% compared with control liver) and cytochrome b_5 reductase (15% compared with control liver) or a decrease in the terminal desaturase enzyme itself.

It has been postulated that SCD is expressed at high concentrations in malignant tissues (Li et al., 1994). To test this hypothesis, a segment of the human SCD cDNA was made from mRNA harvested from healthy adipose tissue by using a primer corresponding to the sequence for rat SCD. Increased SCD mRNA was found in colonic and esophageal carcinomas and in hepatocellular adenoma, but no trend was seen in hepatocellular carcinoma, demonstrating the differences in metabolism of different tumor tissues. An example of SCD expression being associated with tumor tissue was detected by differential display. Genes that are expressed differentially in a chemically induced rat mammary carcinogenesis model were identified by Lu et al. (1997). Seven cDNA fragments were identified and sequenced from the differential display, one of which was 81% homologous to mouse SCD2. The genes identified may represent molecular markers that are helpful in identifying mammary cancer and in aiding in its treatment or prevention.

Fermor et al. (1992) studied the cytotoxicity of fatty acids on normal and malignant cells in hopes of being able to inhibit Δ^9-desaturase activity in cancer cells and increase 18:0 concentrations to inhibit cancerous growth. Both 18:0 and 18:1(n-9) inhibited colony forming abilities of five cell lines tested in culture, but 2- to 3-fold more 18:1(n-9) was needed for a similar response when compared with 18:0. Inhibition of Δ^9-desaturase by using sterculic acid modulated the 18-carbon fatty acid composition by increasing 18:0 by more than 2-fold and decreasing 18:1(n-9) by about 2.5-fold. The changes that were observed were not enough to modify cell viability; furthermore, cytotoxic effects of 18:0 or 18:1(n-9) were no different for cancer cell lines or non-neoplastic cell lines.

de Bravo et al. (1996) studied the effects of lovastatin on fatty acid desaturase activities in a human lung mucoepidermoid carcinoma grown in the legs of nude mice.

Despite nearly a 50% decrease in hepatic cholesterol concentration, there was not a significant decrease in tumor cholesterol. Phospholipid content of lovastatin-treated mice liver decreased but did not change in the tumor tissue, but the phospholipid to cholesterol weight ratio was significantly increased in both tissue of treatment mice. Tumor Δ^9- and Δ^5-desaturase activities increased in treated mice, but Δ^6-desaturase activity did not change. The fatty acid composition of tumor tissue, however, did not reflect the changes in desaturase activity as measured *in vitro*. Despite the failure of lovastatin to decrease fatty acid desaturase in tumor tissue, there is still potential of modulating the fatty acid metabolism of tumor cells, via changes in fatty acid or eicosanoid synthesis to change the rate of growth or even cause cell arrest.

It has been demonstrated that malignant cells can be growth inhibited *in vitro* by 18:2(n-6) and 18:3(n-6). Some types of malignant cells, however, lose Δ^6-desaturase activity, thus requiring 18:3(n-6) as an essential fatty acid (Gardiner and Duncan, 1991). In this study, BL6 melanoma cells, which have no Δ^6-desaturase activity, attained more mass when grown in mice fed 18:3(n-6) than in mice fed 18:2(n-6), indicating a possible negative effect of consuming 18:3(n-6) with respect to some forms of tumor development.

Diabetes

Diabetes mellitus alters fatty acid metabolism, possibly giving rise to many of the secondary disease states observed in diabetic patients. To better understand lipid metabolism in diabetic rats, Δ^6- and Δ^9-desaturase activities were measured in kidneys and livers of control and streptozotocin-induced diabetic rats (Clark and Queener, 1985). The liver

enzyme results were consistent with what was expected in that the Δ^9-desaturase activity in diabetic rats was decreased severely relative to that of control rats, with no change in Δ^6-desaturase activity between treatment groups. In kidney tissue, however, there was an increase in Δ^6-desaturase activity in diabetic rats with no change in Δ^9-desaturase activity. Further, it was determined that, with added fatty acyl-CoA synthetase, Δ^6-desaturase activity in renal plasma membranes from control rats increased to be equal to the activity measured in diabetic rats. Direct measure of fatty acyl Co-A synthetase activity also indicated increased activity in diabetic rat kidney. The authors concluded that the two desaturases are regulated differently in different tissues and that the two tissues are affected differently by the induction of diabetes. In some tissues such as kidney, the rate of fatty acid activation may be limiting.

The hypothesis that available fatty acid precursors for thromboxane synthesis were different in platelets of human diabetics and non-diabetics was tested to explain the increased incidence of microangiopathy in diabetics (Jones et al., 1986). Platelet fatty acid composition was measured in normal, insulin-dependent diabetics, and non-insulin-dependent diabetics. There was an inverse correlation between 18:2(n-6) and 20:4(n-6) in healthy men, but no such relationship existed in diabetic patients. The authors propose that the increased thromboxane synthesis and impaired Δ^5- and Δ^6-desaturases in diabetics deplete the 20:4(n-6) supply from platelets. However, more details of exactly which catabolic steps are inhibited must be obtained before knowing which fatty acids to supplement in attempting to correct this metabolic anomoly.

Bassi et al. (1996) induced ketosis in insulin-dependent diabetic humans and measured fatty acid composition of serum phospholipids as an indicator of fatty acid

desaturase activity. Initial fatty acid profiles between diabetic and control patients were not different. However, after a period of ketosis, where glucose, ketones, and free fatty acid concentrations were elevated and insulin was decreased, 20:4(n-6) and all (n-6) acids as a group were decreased. There was a significant inverse correlation between plasma glucose and plasma 20:4(n-6) concentrations. The change from the baseline ratio of 20:4(n-6) to 20:3(n-6), an indicator of Δ^5-desaturase activity, was significantly and inversely correlated with hemoglobin A_{1c} concentrations. Both short-term increased utilization of 20:4(n-6) and changes in desaturase activities could explain the differences in 20:4(n-6) concentrations. The difference noted in the 20:4(n-6) to 20:3(n-6) ratio before and after ketosis and the correlation of that change with hemoglobin A_{1c} would indicate long-term metabolic control.

Obesity

The regulation of hepatic SCD1 in ob/ob mice fed differing amounts of energy and types of fatty acids was studied (Enser and Roberts, 1982). The ob/ob mice had a 6.5-fold increase in hepatic SCD1 activity compared with lean controls when both were allowed *ad libitum* feed intake. This difference can be explained simply by increased caloric intake by the ob/ob mice because when the ob/ob mice were pair-fed to match the feed intake of the lean controls, their SCD1 activity decreased and became more under the regulation of the type of fat consumed. Not all the difference can be explained by intake, however, because, during a 72-hour starvation, SCD1 activity decreased 99% in lean controls but only decreased 89% in the ob/ob mice. The authors conclude that because of increased *de novo* fatty acid synthesis in ob/ob mice, the 18:2(n-6) that is normally available to regulate SCD1 expression is diluted out by the endogenous saturated fatty acids. This contradicts the

conclusions of Jeffcoat et al. (1979) who proposed that inhibition of fatty acid synthesis occurred through an initial decrease in Δ^9-desaturase activity, leading to a decrease in fatty acid synthesis *de novo*.

Blond et al. (1989) measured hepatic microsomal Δ^5- and Δ^6-desaturase activities in obese Zucker rats (fa/fa) at 6, 9, and 12 weeks of age. In general, obese rats had similar Δ^6-desaturase activity and lesser Δ^5-desaturase activities compared with control rats. The Δ^5-desaturase activity increased 47% in the lean rats during the study compared with only a 10% increase for the obese rats. This change could be accounted for by a increase in K_m of the desaturase in the obese rats at 12 weeks compared with that of the control rats. The *in vitro* measures predicted the tissue fatty acid composition with the 20:4(n-6) to 20:3(n-6) ratio being 5-times greater in lean control rats with little difference noted in the 20:4(n-6) to 18:2(n-6) ratio. These results demonstrate the differences between obese animal models, where obese mice (ob/ob) have increased Δ^5- and Δ^6-desaturase activities and more 20:4(n-6) in hepatic membranes than do lean control mice.

Phinney et al. (1993) wanted to correct the abnormally decreased concentration of phospholipid 20:4(n-6) in obese Zucker rats by feeding 18:3(n-6). One proposed problem noted in obese humans and rats is a great decrease in 20:4(n-6), which causes speculation about inadequate desaturase systems. Dietary 18:3(n-6) normalized 20:4(n-6) in phospholipids of obese rats, caused a decrease in food intake, and resulted in a decreased amount of body fat. The other (n-6) metabolites of 18:2(n-6) were similar to those of control rats, indicating a sufficient desaturase system but an altered metabolism of 20:4(n-6) in the obese rats. In fact, they found a greater amount of cholesteryl arachidonate in the serum and hepatic tissue of obese rats than in the lean control rats. They caution direct extrapolation of

these data in utilizing supplemental 18:3(n-6) as a human diet compound because of the risk associated with elevated 20:4(n-6) in tissues.

A review by Pan et al. (1994) described the effects of types of dietary fats and their effect on membrane phospholipids as they relate to obesity. Increased Δ^9-desaturase activity has been associated with obesity in several animal studies (chickens, Legrand and Hermier, 1992; rats, Wahle and Radcliffe, 1977; pigs, Kouba et al., 1997; humans, unpublished data noted in Pan et al. 1994). Not as many data are available for Δ^5- and Δ^6-desaturase activities related to obesity. Indirect evidence provided by Nakamura et al. (1994) indicates, in cases of insulin resistance, such as in chronic alcoholism, a decrease in Δ^5- and Δ^6-desaturase expression. Borkman et al. (1993) concluded that the strongest single correlate of insulin action was that of Δ^5-desaturase activity as monitored by tissue ratios of 20:4(n-6) to 20:3(n-6). The authors propose that decreased unsaturation because of lesser expression of fatty acid desaturases would lead to less fatty acid oxidation, and therefore more triacylglycerol storage.

Jones et al. (1996) determined stearoyl-CoA expression in adipose tissue, a major site of SCD expression and lipid metabolism, in lean and obese Zucker rats fed control (12% corn oil) or PUFA-enriched (48% corn oil) diets. The pattern of increased desaturase expression in obese animals and a repression of expression with increased dietary PUFA in both lean and obese rats mimics data observed for hepatic SCD expression in other studies with rodents and other animal species. It is not known if the same control mechanisms are acting in adipocytes as compared with hepatocytes. It was noted by Shillabeer et al. (1990) that, because of the delay in deliver of dietary fatty acid to the adipose tissue compared with

delivery time to the liver, there will be a delayed response time in SCD expression in adipocytes compared with hepatocytes when dietary lipids are fed.

Fatty acid composition of several blood lipids were analyzed in 22 obese and 25 aged-matched control children averaging 13.7 years of age (Decsi et al., 1996). By comparing pre- and post-Δ^6-desaturase metabolites, the obese children had more Δ^6-desaturase activity than did the aged-matched controls. Plasma glucose concentrations were related inversely to 20:4(n-6) in phospholipid and sterol esters and to the 20:4(n-6) to 20:3(n-6) ratio in phospholipid and sterol esters. A logical conclusion is that increased concentrations of longer chain (n-6) metabolites of 18:2(n-6) is probably a result of increased Δ^6-desaturase activity via elevated fasting insulin in obese children.

Hypertension

Correlations between hypertension and membrane fatty acid composition have been noted for some time (Watanabe et al., 1989). Narce and Poisson (1995) used a spontaneously hypertensive rat model to study the effects of hypertension and aging with respect to Δ^5- and Δ^6-desaturase activities because increased dietary supply of 18:2(n-6), a prostaglandin precursor, can attenuate blood pressure. In this study, the ratio of 18:2(n-6) to 20:4(n-6) in total liver lipids increased in the hypertensive rats with aging (7 weeks of age to 13 weeks of age) but not in the control rats. Delta-5 and Δ^6-desaturase activities were measured from liver microsome preparations, and, as predicted from the fatty acid composition both Δ^5- and Δ^6-desaturase activities were decreased in hypertensive rats. At 13 weeks of age, both desaturases in hypertensive rats were less than that measured in controls. In later studies by

Narce et al. (1995) where rats were allowed to age further, Δ^6-desaturase activity of the hypertensive and normal rats equalize and approach the activity observed in control rats at 1 month of age. The initiation of hypertension in this rat model may be via change in eicosanoid balance during the growth phase of the rats, the time when hypertension is manifested.

de Catalfo and de Gómez Dumm (1996) found a decrease in hepatic Δ^5- and Δ^6-desaturases and long-chain fatty acyl-CoA synthetase activities in spontaneously hypertensive rats compared with controls. In testis, the thioesterification and Δ^5-desaturase activities also were inhibited in hypertensive rats. Membrane fatty acid composition revealed the changes measured by *in vitro* enzyme activity. These authors also proposed that increases in eicosanoid availability via increases in desaturase activities could explain the differences between spontaneously hypertensive and normal rats.

Dietary supplemental fatty acids that bypass Δ^5- and Δ^6-desaturases have been demonstrated to attenuate pressor and tachycardic response in stressed normotensive and hypertensive rats (Mills et al., 1985). Isolation stress in spontaneously hypertensive and Wistar Kyoto (control) rats caused a decrease in Δ^5- and Δ^6-desaturase activities in liver microsomes (Mills et al., 1994). In the same paper, an experiment utilizing essential fatty acid-deficient Sprague-Dawley rats injected with epinephrine and gavaged with 18:2(n-6) showed decreased 20:4(n-6) in plasma lipids while 18:2(n-6) was increased. This observation indicates a dose response of epinephrine on inhibition of desaturases that are required to synthesize 20:4(n-6) from 18:2(n-6) in rats.

Russo et al. (1997) compared fatty acid composition of plasma and erythrocyte membranes and zinc status of hypertensive and control patients to understand the association

of fatty acid desaturase activity and hypertension. Greater zinc concentrations and ratios of fatty acids in plasma that are indicative of greater desaturase activity were observed in hypertensive compared with control patients. The increased desaturase activity will provide more eicosanoid precursors, which could explain the association between fatty acid desaturase activity and hypertension. The direct association between hypertension and desaturase activities remains to be seen, but this study provides good evidence for a strong correlation despite contradictions to hypertensive rodent models.

General Disease

Stearoyl-CoA desaturase has been associated with all three studies discussed in this section of the review. Kaput et al. (1994) have been studying genes that are regulated by great amounts of dietary fat in an attempt to understand disease processes associated with increased dietary fat intake. Two genes identified by using this method, originally designated Lfm-1 and Lfm-2, have been identified as an F_1F_0-ATPase-like gene and SCD, respectively. Lfm-1 has a restriction-length polymorphism (RFLP) on chromosome 8 of mice associated with atherosclerosis. This general approach of subtractive hybridization and +/- screening (Elliott et al., 1993) has the potential of unlocking genes expressed during dietarily induced disease states and then predicting the risk of diet-related diseases in individuals.

Paisley et al. (1996) fed BALB/cHnn mice diets containing 3, 10, or 20% corn oil for 2 weeks and then studied hepatic Δ^9-desaturase expression following food depravation and refeeding. This effort is part of the scientific movement to understand gene expression in normal and disease states as a way of predicting health status. As would be expected, the

mice fed more corn oil had less SCD mRNA. In the same study it also was noted that starvation greatly elevated the SCD1 expression whereas refeeding suppressed activity.

Differential disease induction by dietary fat in two strains of mice was studied (Park et al., 1997). One strain, C57BL/6J, is highly susceptible to atherosclerosis, diabetes, and disorders of lipid metabolism while BALB/cByJ mice served as the healthy controls. Both types of mice were fed diets containing either 4% or 20% coconut or corn oil for 4 months. The BALB/cByJ mice grew faster on all four diets. Analysis of the SCD promoters of both strains revealed no difference in DNA sequnences, but northern analysis revealed SCD1 expression was greater in C57BL/6 mice than in BALB/c mice. While the BALB/c mice responded to increased corn oil or coconut oil by down-regulating SCD1 expression, the C57BL/6 mice responded nicely by down-regulating SCD1 expression with an increase in corn oil but had high rates of expression with either 4% or 20% coconut oil diets. These data will be used in future research to study the heritability of certain diseases linked to abnormal lipid metabolism.

Gamma-Linolenic Acid Supplementation

In 1993, Horrobin wrote a review on the role of Δ^6-desaturase in health and disease. In this article, he addressed the idea of 18:3(n-6) supplementation of diets to overcome shortfalls in the Δ^6-desaturation of 18:2(n-6). Desaturation and elongation of 18:2(n-6) is crucial because 18:2(n-6) has little value metabolically other than supplying energy. The products of 18:2(n-6) desaturation, however, have increasing biological potency as the molecule becomes longer and less saturated. The longer chain acids are precursors to the eicosanoids, which regulate many aspects of physiology such as reproduction and immune

mediation. Topics of medical interest associated with dietary supplemental 18:3(n-6) include inclusion of post-Δ^6-desaturase metabolites in infant formula and supplementation of 18:3(n-6) to bypass Δ^6-desaturase in treating the symptoms of atopic eczema and diabetes.

Karlstad et al. (1993) studied the effect of 18:3(n-6) addition to parenteral feeding of control and injured rats. By supplying increasing amounts of 18:3(n-6) to injured rats, they observed a favorable reduction in the ratio of thromboxane B_2 to 6-keto-prostaglandin $F_{1\alpha}$. These results were achieved when the Δ^6-desaturation of 18:2(n-6) was bypassed with additional dietary 18:3(n-6) that increased 20:3(n-6), the precursor to prostaglandin E_1, an anti-inflammatory prostaglandin. This treatment also attenuated 20:4(n-6)-derived prostaglandins. Arachidonic acid concentration in plasma did not increase with increased amounts of 18:3(n-6). Gamma-linolenic acid conversion to 20:3(n-6) actually decreases the amount of 20:4(n-6) formed because, as 20:3(n-6) is being converted to the one series prostaglandins, there is competition for cyclooxygenase conversion of 20:4(n-6) to the inflammatory prostaglandins.

Heart

Possible new predictive risk factors for coronary heart disease (CHD) have been observed in a health survey study by Öhrvall et al. (1996). Two-thousand-three-hundred and twenty-two 50-year-old men were studied, and it was discovered that participants with lower proportions of 14:0, 16:0, 16:1(n-7), and 20:3(n-6) in plasma cholesteryl esters were at less risk for CHD than those with higher amounts of 18:2(n-6) cholesteryl ester. Middle aged men who develop CHD have a low 20:4(n-6) to 20:3(n-6) ratio of plasma cholesteryl ester, indicating a possible decrease in Δ^5-desaturase activity in individuals at risk.

Liver

Both Δ^5- and Δ^6-desaturase specific activities increased in liver of rats 3 to 7 days after 65% hepatectomy (Carreau et al., 1981). The activity of both enzymes increased to match total desaturation capacity of the intact livers in sham-operated control rats. On the other hand, liver cirrhosis in humans, regardless of the origin, caused a 50% decrease in Δ^6-desaturase activity compared with control livers (Biagi et al., 1990). Despite the large change in enzyme activity, fatty acid composition of the liver microsomal membranes was not different. This response is a good demonstration of the ability of organs to maintain membrane integrity even in the advent of dire circumstances.

Neurology

Carreau et al. (1979) measured Δ^9-desaturase activity in brain microsomes from normal mice and mice with genetic defects causing neurological abnormalities. Up until this study, the existence of Δ^9-desaturase activity in brain tissue was controversial. This experiment demonstrated Δ^9-desaturase activity in neural tissue and that the desaturase was not as active in the diseased mice during myelination, a period of rapid brain growth and membrane synthesis.

DeWille and Farmer (1992) detected decreased expression of genes known to be critical for neuronal myelination in mice of the quaking phenotype. Neural mRNA for the major myelin proteins, proteolipid protein, and myelin basic protein, along with SCD, LDL-receptor, and ApoE, were all greatly decreased in 23-day- old mice with the quaking phenotype compared with control mice. Not only is MUFA synthesis down-regulated during a critical time of brain growth, but critical proteins that become part of the neural tissue and

proteins such as the LDL-receptor and ApoE that help sequester and transport lipids are suppressed as well.

DeWille and Farmer (1993) compared brain SCD2 mRNA to hepatic SCD1 mRNA in rat pups nursed by mothers fed a control (5% corn oil) or an EFAD diet (5% coconut oil). The corn oil diet was formulated to just meet the essential fatty acid requirements of the nursing pups. There was a 2-fold decrease in brain SCD2 mRNA when dams were fed an EFAD diet, whereas SCD1 mRNA in liver increased 29-fold. While modulation of hepatic expression by diet has been demonstrated frequently, this experiment is the first noted example of dietary lipids controlling a gene encoding a lipogenic enzyme in brain. The coding sequences for SCD1 and SCD2 are similar, but the promoter for each is quite different, containing only a few regions of homology. Both SCD1 and SCD2 promoters contain C/EBP consensus binding sites. C/EBP-α, β, and δ are expressed in liver, whereas the δ form is the only major C/EBP expressed in brain. Expression of any of the C/EBPs, however, did not change in either tissue with either diet, giving rise to the question of post-translational modification of C/EBPs for modulation of activity.

Cultured fibroblasts from skin-punch biopsies from schizophrenic patients, bipolar patients, and normal controls were grown in culture and analyzed for phospholipid fatty acid composition. Fatty acid composition of controls and bipolar patients did not differ, but those with schizophrenia had lesser amounts of 22:6(n-3) as well as less total (n-3) fatty acids. Arachidonic acid did not differ across groups, indicating a deficiency in Δ^4-desaturase activity among schizophrenic patients (Mahadik et al., 1996a). In a later study by Mahadik et al. (1996b), fibroblasts grown from skin-punch biopsies were provided 18:2(n-6) and 18:3(n-3) separately in culture. Uptake and incorporation of these two acids into phospholipids were

not different between patients and controls. Their conversion to longer chain-unsaturates, however, indicated a defect in Δ^4-desaturase in schizophrenic patients.

Fatty acid composition analysis of pathologically proven Alzheimer's diseased brains and age-matched controls were obtained for study (Nakada et al., 1990). In the diseased brains, 18:2(n-6) was elevated from 6- to 25-fold in the various phospholipid classes compared with those of controls. At the same time, 20:4(n-6) and 22:4(n-6), metabolites from 18:2(n-6), were unchanged to 12-fold less in the various phospholipid classes of diseased patients. In the (n-3) series, 22:6(n-3) was unchanged to 3-fold less in the diseased brains. These findings link the pathology of Alzheimer's disease with defective fatty acid desaturase systems.

Toxins

Carreau et al. (1980) quantified Δ^6- and Δ^9-desaturase activities in rat liver after carbon tetrachloride poisoning to learn about desaturase activity recovery during liver regeneration. Delta-9-desaturase activity decreased to a low of 24% of control and recovered to 94% of control after 14 days of recovery. Delta-6-desaturase responded more rapidly to the toxin and did not recover as fully. Delta-6 desaturase activity already had decreased 30% at 3 hours, attained a minimum of 37% of control activity at 12 hours, and rebounded to 72% activity at 14 days. The supporting proteins, NADH-ferricyanide reductase, and NADH-cytochrome c reductase activities were not as severely depressed as that of the terminal desaturases.

Borlakoglu et al. (1991) noted a great increase in 20:4(n-6) in hepatic microsomal membranes of rats and pigeons dosed with Aroclor, a polychlorinated biphenyl. Along with

the significant increase in cytochrome P_{450} concentration and increases in activities of associated enzymes was an increase in Δ^6-desaturase activity (40-fold in pigeons and 10-fold in rats), leading the authors to question if Δ^6-desaturase is actually a P_{450}-dependent monooxygenase. When cytochrome P_{450} is extrapolated to zero, it is coincident with zero desaturase activity.

Desaturases in Other Tissues

Fatty acid desaturases usually are associated with liver, adipse, and neural tissues. Other tissues that have been studied with respect to fatty acid desaturation include mammary, intestinal, kidney, fetal, and lens tissues. This section surveys the various tissues not covered in other sections of the review.

Kinsella (1972) assayed SCD activity in bovine mammary microsomes and established that the cofactor requirements are the same as those for hepatic SCD, but little or no SCD activity can be detected in tissues obtained from non-lactating cows. Desaturation of 18:0 was proposed as a limiting factor in milk triacylglycerol synthesis *in vivo* and the theory was tested *in vitro*. The affinity of the transacylases for activated 18:0 or 18:1(n-9) differed, with glyceride acyl transferase preferring 18:1(n-9) and phosphatidyl acyl transferase preferring 18:0. When SCD was inhibited or stimulated *in vitro*, the rate of triacylglycerol synthesis was inhibited or stimulated in a parallel fashion. This *in vitro* response is suggestive that any factor that affects the activity of SCD such as availability of activated precursor, NADH concentration, or concentration of SCD would limit milk triacylglycerol synthesis.

Delta-9-desaturase from lactating rat mammary gland and liver was studied by Calabro et al. (1982). They found that mammary tissue had 1.25- to 25-fold less terminal desaturase activity than did liver tissue. When purified Δ^9-desaturase was added to mammary microsomes, there was a 55-fold stimulation of desaturase activity. Cytochrome b_5 addition had no effect on activity, but when both cytochrome b_5 and Δ^9-desaturase were added, there was a 200-fold increase in activity. These results suggest that the majority of monounsaturated lipids incorporated into milk lipids in rats are probably dietary or hepatic in origin.

Bandyopadhyay et al. (1995) has documented a 4-fold increase in specific activity of Δ^5-desaturase in intact mammary fat pads of pregnant mice compared with lactating mice. This change is a good example of how mammary adipose tissue behaves differently during pregnancy and lactation as it is not dedicated to sequestration and accumulation of dietary lipids but rather to production of stimulatory type molecules needed to initiate and support lactation, such as eicosanoids.

Intestinal cell membrane integrity is of utmost importance in maintaining absorptive capabilities. It is well documented that membrane fatty acid composition can greatly affect membrane fluidity and therefore greatly change its physiochemical character. Garg et al. (1988) studied Δ^6- and Δ^9-desaturase activities in microsomes prepared from rat jejunum, ileum, and liver. Delta-9-desaturase specific activity was about 50% in jejunum and ileum compared with liver, whereas Δ^6-desaturase activity was about 50% in the jejunum and 25% in the ileum compared with liver. While it is possible for the enterocytes to obtain much of the fatty acid required for membrane growth from recovery of bile-derived phospholipid fatty acids, the enterocytes are also capable of *de novo* synthesis of PUFAs.

Keelan et al. (1997) studied fatty acid desaturases in portions of the jejunal villus from rats fed PUFA- or saturated fatty acid-enriched diets. Previously, they had noted changes in phospholipid fatty acid composition in the upper versus the lower segments of the villi. To determine if these changes were the result of differential desaturase expression, the villi were isolated in four fractions from distal to proximal regions for analysis. Delta-5-desaturase activity was greatest in the upper fractions compared with the lower fractions and was decreased when dietary saturated fatty acids were fed compared with PUFAs. Delta-6- or Δ^9-desaturase activities did not change because of dietary manipulation or with location within the villus. Despite the change in Δ^5-desaturase activity, there were no differences in fatty acid composition that reflected this change.

Delta-5-desaturase activity was confirmed in rat kidney microsomes by Irazú et al. (1993). The enzyme has characteristics similar to those described for hepatic Δ^5-desaturase. The concentration of Δ^5-desaturase is over 10-times greater in liver compared with kidney tissue. Kidney Δ^5-desaturase also differed from hepatic Δ^5-desaturase in that it is not further activated by addition of cytosolic preparations from liver or kidney cells. Activity of Δ^5-desaturase in kidney cells indicates that this organ is capable, at least in part, of satisfying its own eicosanoid needs.

Salviati et al. (1979) noted that NADH-cytochrome c reductase activity of sarcoplasmic reticulum fragments, a measure of NADH-cytochrome b_5 reductase-cytochrome b_5, was much greater in slow (red) muscles than in fast (white) muscles. They also note an inverse relationship between Ca^{2+}-transport and electron transport activity of

different membranes. Their data support that decreased Δ^9-desaturase activity is restricted because of terminal desaturase enzyme in slow muscles.

Unesterified fatty acids, the major source of plasma lipid supply for the developing fetus, in the plasma of ewes is much more saturated than are the fatty acids found in total lipids of the placenta and fetal plasma (Shand and Noble, 1979). These differences correlate with an active desaturase system in the placentae of ewes. Placental Δ^9-desaturase activity was found to be 4-fold greater than that of newborn lamb liver, double that of 8-day-old lamb liver and 30% higher than adult liver, whereas placental Δ^6-desaturase was 47-, 8-, and 12-times greater in placentae than in newborn liver, 8-day-old liver, and adult liver. It is now clear that the placenta not only acts as a barrier, allowing appropriate nutrients to pass into the developing fetus, but also as a processor of fatty acids, modifying highly saturated maternal blood lipids into longer chain, more unsaturated acids for the developing fetus. This conclusion directly contradicts the functions of the human placenta where no Δ^5- or Δ^6-desaturase activities were found, but rather an impressive but declining activity of these two enzymes in fetal liver from 18- to 22-weeks of gestation was observed (Chambaz et al., 1985). In humans, the differential fatty acid composition of the fetus and the mother must be explained by preferential uptake of fatty acids across the placenta and/or active fetal elongation and desaturation of fatty acids. Poisson et al. (1993) evaluated human new born liver for Δ^5- and Δ^6-desaturase activities and determined that both desaturases were present but they were less concentrated than that found in adult liver. Also, there was substrate inhibition by the (n-6) fatty acids but not by the (n-3) fatty acids.

^{14}C-Stearic acid, when incubated with rabbit lens tissue in culture, is β-oxidized, the label is transferred into 12-, 14-, and 16-carbon fatty acids as well as into cholesterol, it is Δ^9-desaturated, and it is elongated (Albers-Jackson and Bunch, 1982). The resulting acids also were incorporated into triacylglycerols and phospholipids. These results demonstrate that lens has appreciable metabolic and catabolic ability and is not completely reliant upon the aqueous humor as a supplier for all fatty acid precursors and metabolites.

Molecular Biology

Animal Systems

Thiede et al. (1986) isolated the cDNA for rat liver SCD by inducing the expression with alternate periods of fasting and feeding a high-carbohydrate diet, performing primer-extension of cDNA, and analyzing overlapping fragments. The resulting 4,689 bp cDNA sequence includes a 1,074 bp open reading frame coding for a 358 amino acid enzyme of molecular weight 41,400 daltons. The large region of 3' untranslated region has been identified as coming from one exon by Southern blot analysis. Comparison of cyanogen bromide sequence analysis of the purified enzyme and total amino acid analysis confirmed the cDNA as being that for SCD. The protein is comprised of 62% hydrophobic amino acids.

Strittmatter et al. (1988) expressed rat liver SCD in *E. coli*. One construct contained the sequence for amino acid residues 3-358 and the other for residues 27-358. Both proteins were associated almost exclusively with membrane fractions of cell homogenates. Posttranslational iron addition to the apoprotein can be achieved *in vitro* or by adding iron during induction of the cDNA. Both proteins expressed activity when appropriate cofactors were added in an assay system. Both expression constructs provided equal activity,

indicating that the region that was deleted in the second construct was not crucial to proper membrane insertion, iron sequestration, or catalysis. The deleted region is unique in that it contains an unusually high content of hydroxylated residues

Promoters

A rat genomic library was screened to determine the gene structure of SCD1 and SCD2 (Miharra, 1990). The upstream regulatory regions differ greatly between the two forms. Stearoyl-CoA desaturase-one is organized with six exons and five introns spanning about 15 kb and coding for an mRNA with an open reading frame of 1074 bp. The 5'-flanking region of SCD1 contains sequences that are homologous to the FSE2, a negative regulatory element of gene expression during differentiation. The tissue distribution of SCD1 and SCD2 mRNAs differs greatly. Stearoyl-CoA desaturase-1 is in adipose tissue and can be induced in liver, whereas SCD2 is in brain, kidney, and testis.

Ntambi (1995) reviewed the differential expression of SCD1 and SCD2 and noted that the two forms probably arose from a single gene because the coding regions are conserved (87% amino acid identity) and the 5'-promoter region contains similar elements. In the promoter region, the two genes share a 146 bp sequence that is 77% identical and contains two CCAAT boxes.

The CCAAT/enhancer binding protein (C/EBP) is central in activating transcription of several genes during preadipocyte differentiation including SCD. Several lines of evidence that implicate C/EBP as determined by Christy (1989) include: 1. C/EBP binds to SCD1, GLUT4, and aP2 promoters, 2. C/EBP is expressed just prior to SCD1, GLUT4, and aP2, 3. Cotransfection of C/EBP with reporter genes linked to the promoters of the genes

mentioned above result in increased amounts of reporter product, and 4. Mutations in the C/EBP binding sites in the promoter regions result in a decreased amount of *trans* activation.

Swick and Lane (1992) identified a transcriptional repressor that binds to the promoter region of SCD2 and is only expressed before preadipocytes are allowed to differentiate into adipocytes. They termed the binding site PRE (preadipocyte repressor element). The PRE was located –435 to –410 relative to the SCD2 start codon. By using Southwestern blotting, the PRE binding protein was found to be present in preadipocytes and HeLa cells but not present or active in adipocytes. When the PRE was inserted upstream of the SV40 enhancer/promoter, the reporter gene was expressed in a pattern similar to that for SCD2 in preadipocytes, adipocytes, and HeLa cells.

Sterol-regulatory element binding protein-1a (SREBP-1a) is a membrane-bound protein associated with the nuclear envelope. Upon sterol-depletion-dependent proteolysis, SREBP-1a is cleaved, creating a soluble NH_2-terminal domain that enters the nucleus and activates transcription of several cholesterol and fatty acid biosynthetic genes. Shimano et al. (1996) overexpressed the soluble NH_2-terminal fragment of SREBP-1 in transgenic mice. While serum lipids did not change, hepatic lipid synthesis was greatly up-regulated, resulting in liver enlargement as triacylglycerols and cholesterol accumulated. One of the lipogeneic genes activated was SCD.

The promoter for SCD1 contains several *cis*-DNA elements that mediate responses to PUFAs (Waters et al., 1997), thyroid hormone, (Waters et al., 1997), cAMP analogs (Casimir et al., 1996), and PPAR (Miller et al., 1996). The transcription factor C/EBPα is known to bind to the SCD1 promoter between –60 and –80 (Christy et al., 1989). Region –253 - +30

contains *cis*-DNA elements that respond to the AP-2-mediated cAMP induction of SCD1 (Casimir et al., 1996).

The PUFA response region of SCD1 was localized by using a chimeric 5'-flanking region fused to a reporter gene (Waters et al., 1997a). When the entire 4.3 kb SCD1 putative promoter was used, 18:3(n-3) repressed transcription by 60% and 20:4(n-6) and 20:5(n-3) by 75%, but no change was observed with 18:0 addition. Progressively smaller SCD1 chimeric constructs revealed a 237-bp sequence that was responsible for PUFA regulation in the proximal promoter. Mobility shift analysis identified a 60-bp sequence that bound proteins from nuclear extracts from 3T3-L1, Hep G2, and mouse liver cells. The 60-bp sequence, which is also present in the SCD2 proximal promoter, confers PUFA responsiveness to reporter constructs. This observation indicates that SCD1 (liver) and SCD2 (adipose) may share common transcription regulatory mechanisms.

Stearoyl-CoA desaturase has been used as a model for studying the patterns of lipogenic gene expression. The way SCD1 is down-regulated and up-regulated by PUFA and insulin, respectively, is similar to the way other lipogenic genes respond (Ntambi, 1992). An example of SCD1 not responding along with the other lipogenic genes is the up-regulation of hepatic SCD1 by retinol palmitate, with no change in fatty acid synthase expression (Miller et al., 1997). Other examples of SCD1 not responding as a lipogenic gene include thyroid hormone repression (Waters et al., unpublished data) and peroxisome proliferator induction (Miller and Ntambi, 1996). Because thyroid hormone represses retinol-elevated SCD1 expression and because peroxisome proliferator-elevated SCD1 mRNA are unaffected by administration of thyroid hormone, SCD1 regulation evidently is under control of

heterodimerization with the retinoid X receptor and the peroxisome proliferator regulation occurs through a different mechanism (Miller et al., 1997).

Singh and Ntambi (1998) report the necessity of nuclear factor 1 (NF1) for SCD1 expression during preadipocyte differentiation. Region –114 to –86 is essential for transcriptional activation of SCD1 during differentiation and contains the sequence TGGCA, a known NF1 binding site. By using chimeric promoter/reporter constructs, it was determined that the NF1 binding site was necessary for differentiation-induced expression of SCD1 by both NF1 and cAMP-response. Gel-shift assays confirm that mutated forms of the NF1 binding sequence do not associate with NF1. Also discovered was an additional protein in the NF1 family that is present only after preadipocyte differentiation that is specific for NF1 sequence as based on competition assays. The authors believe that this protein is modified during differentiation because cycloheximide does not inhibit binding to the DNA during addition of the differentiation mix. Evidently, NF1 interacts with other regulatory elements during differentiation to induce changes in SCD1 expression.

Genetics

Birds

Avian lipid metabolism is markedly different from that in mammalian systems because of the anatomical site of de novo lipid synthesis. In birds, the liver is the predominant tissue of *de novo* fatty acid synthesis and adipocytes are primarily utilized for storing triacylglycerol. In the pig, however, adipocytes are primary sites of both synthesis and storage. Other animal species synthesize fatty acids in both liver and adipose to varying degrees.

Joshi et al. (1977) determined that hepatic microsomes from 20-day-old chicken embryos or 1-day-old chicks contained ample cytochrome b_5 and cytochrome b_5 reductase to support Δ^9-desaturase activity, but no detectable terminal Δ^9-desaturase. Therefore, purified desaturase could be added to microsomal preparations from young chicks and a functionally complete desaturase system would be available for study. This *in vitro* system demonstrated the lack of Δ^9-desaturase in embryonic chicks and also provided an inexpensive source of supporting enzymes and proteins to assay Δ^9-desaturase for other experiments. Legrand et al. (1994) studied primary chicken hepatocytes in culture and determined that they respond to insulin and PUFAs the same as do rodent hepatocytes. The authors noted a 30-hour delay in maximal SCD activity when hepatocytes were isolated from fasted chickens. This methodology should be foundational to further studies of hormone or metabolite regulation of SCD or of other genes expressed in chicken hepatocytes. Recently, SCD1 from chicken has been cloned, and Fillon et al. (1997) have reported that chicken SCD1 has been mapped to chromosome 6 (R-band 6q14).

Because fatty liver is sometimes a problem in laying hens, resulting in fatty liver hemorrhagic syndrome and even death, mechanisms to maintain triacylglycerol flux from the liver in the form of VLDL particles are important to understand. Hermier et al. (1996) used estrogenized young male chickens to study the relationship between fatty acid desaturation, hepatic lipid storage, and VLDL secretion. Hepatic SCD was 2-fold higher in estrogenized chickens than in control chickens. Stearoyl-CoA desaturase induction was noted by increased MUFA concentration in VLDL, liver, and liver microsomes. The MUFA content of VLDL fatty acids was 55% whereas the MUFA content of liver fatty acids was 50%. This disparity in MUFA concentration indicates the preferential export of monoenes and provides

the opportunity to protect the liver from triacylglycerol accumulation and resultant steatosis. This protection theory was supported when Legrand et al. (1997) inhibited Δ^9-desaturase activity in cultured chicken hepatocytes and were able to decrease triacylglycerol secretion rates by greater than 4-fold. The authors had noted the increased 18:1(n-9) content in secreted triacylglycerols (VLDL) compared with intracellular triacylglycerols. When 18:1(n-9) was added to the cultures that had inhibited Δ^9-desaturase activity, there was an increase in triacylglycerol secretion rate, but it did not return to the rate of the control cells. In summary, the authors suggest that this could be the mechanism linking increased Δ^9-desaturase activity with excessive over-fattening in chickens.

Ruminants

Whereas the highly reductive environment in the rumen almost completely saturates dietary PUFA prior to absorption in the ruminants (Ashes et al., 1997), the fatty acid composition of ruminant animals can vary considerably. Ch'ang et al. (1980) determined the genetic effects of sire on five fatty acids in sheep and determined that the weight percentage of 16:0 and the ratio of palmitoleic to 16:0 were highly heritable (0.40 and 0.38, respectively). From this study, it is feasible to consider a genetic approach to altering physical-chemical properties of fatty acids in animal products by selecting for desired desaturase activities.

Chang et al. (1992) fed high 18:1(n-9) sunflower seed to steers to learn about lipid metabolizing enzymes such as SCD and fatty acid elongase. The steers fed increased concentrations of 18:1(n-9) had elevated plasma 18:1(n-9), and 18:1(n-9), 18:0, and myristate were elevated in adipose tissue, probably as a function of the increased lipid in the diet.

While fatty acid elongase was not modulated by diet in tissues sampled, SCD was increased in skeletal muscle, with numerical increases in adipose, liver, and small intestine. There was a 30% decrease in liver 18:0 in cattle fed the high 18:1(n-9) sunflower seed, despite having a 3-fold increase in duodenal 18:0 available for absorption. The authors suggest an adaptive response of the steers, allowing them to up-regulate SCD when excess 18:0 is present.

American Wagyu cattle, the North American counterpart of Japanese Black cattle, have increased concentrations of MUFAs in their tissues compared to other beef cattle breeds. Wilson et al. (1993) noted the identification of an RFLP in the SCD gene in DNA from purebred Japanese Black Cattle. Cameron et al. (1994) proposed that the increased 18:1(n-9) in Wagyu cattle when compared with Black Angus cattle was because of increased SCD expression or activity. However, there were no differences in mRNA quantity or enzyme activity in mature cattle of both breeds. The authors concluded that the differences must occur at earlier stages of development.

Page et al. (1997) fed whole cottonseed to steers in an attempt to depress SCD activity in adipose or liver tissue. The authors believed this could occur because of cyclopropene fatty acids present in whole cottonseed. The steers fed whole cottonseed attained a greater body weight and had greater subcutaneous adipose tissue and marbling because of greater dietary fat intake. Of the biochemical parameters measured, the only difference noted was a suppression in lipogenesis in steers fed whole cottonseed probably because they were fed greater amounts of dietary fat. Delta-9-desaturase activity and adipose cellularity were not changed. Again, the reductive nature of the rumen converted dietary PUFAs into mostly saturated fatty acids before they were absorbed. This occurrence makes

the PUFA regulation of SCD in adipose tissue much different in ruminants than in nonruminants.

Ovine SCD is the only example of a cloned SCD from ruminants. An adipose tissue cDNA library was screened to obtain the clone by using a 1.2 kb rat SCD1 probe. The resultant sequence was 90% homologous with mouse, rat, and human sequences (Ward et al., 1997). By restriction analysis and by screening a genomic library, it was verified that there is only one unique SCD gene in sheep, which is unlike that in the mouse where both SCD1 and SCD2 have been mapped to adjacent sites on mouse chromosome 19 (Tabor et al., 1998).

Rodents

de Antueno et al. (1994) compared the Δ^5- and Δ^6-desaturase activities of four strains of laboratory rats and compared microsomal membrane fatty acid composition to the enzyme activities. There was over a 2-fold difference in enzyme activities when the strains with the greatest and least activities were compared. Despite great differences in activity, these two desaturases were tightly correlated between strains. The greatest activity was observed for the BB-Wistar control rats, with the CR Wistar and Long-Evans strains being intermediate, and Sprague-Dawley rats having the least activity. The authors emphasize that no significant correlations were found between either Δ^5- or Δ^6-desaturase activities as determined by *in vitro* assay and the fatty acid substrates or products as part of the microsomal fatty acid composition.

Ulmann et al. (1994) measured incorporation of 18:2(n-6), 18:3(n-3), and 20:3(n-6), and their metabolites into hepatic microsomal phospholipids of rats fed diets enriched in (n-3) fatty acids (5% fish oil) or (n-6) fatty acids (19% borage oil). Obese Zucker rats, their

lean littermates, and Wistar control rats were used. The majority of the labeled acids were found in the triacylglycerols of obese rats but in phospholipids of lean rats. All phenotypes incorporated more 18:3(n-3) and its metabolites when fed the fish oil diet. The fish oil diet favors the (n-3) fatty acid biosynthesis and incorporation into liver microsomal lipids classes as the (n-6) fatty acids are synthesized and incorporated at a lesser rate. This study demonstrates dietary and genotype effects on several aspects of fatty acid metabolism.

Hughes and York (1985) noted an increase in Δ^5-desaturase and Δ^6-desaturases in liver of young genetically obese (ob\ob) mice. The onset of increased desaturase activity does not occur until weaning. Restricting food intake or giving insulin does not restore the two desaturases to control desaturase activity. Cold acclimation and triiodothyronine, however, decrease Δ^6-desaturase activity to be equvalent to activity observed in control mice.

Pigs

Activity of SCD in adipose and liver tissues from growing Large White and Meishan pigs was studied (Kouba et al., 1997). Both breeds had more SCD activity in adipose tissue than in liver when expressed on a protein basis, but, because of the 10-fold greater concentration of microsomal proteins in liver, when expressed on a tissue weight basis, the liver had more activity. However, the adipose tissue weight is greater than the liver weight, making liver desaturase a minor component of overall fatty acid desaturation in the pig. The specific activity of SCD in adipose was much greater in Large White than in Meishan pigs, and the 18:1(n-9) content of Large White adipose was slightly lower. Hepatic 18:1(n-9) content was higher in Meishan pigs, which had an increased hepatic SCD activity compared with Large White pigs. This observation holds true to other studies that have demonstrated

rats (Wahle and Radcliffe, 1977) and chickens (Kouba et al., 1993) with higher body fat, like the Meishan pig, have increased hepatic SCD activity. But, there was a lack of correlation in adipose SCD activity with 18:1(n-9) content of adipose tissue much as in the study comparing Black Angus and American Wagyu cattle (Cameron et al., 1994). Some of the differences in adiposity of strains of animals within the same species may arise from increased Δ^9-desaturase activity providing more 18:1(n-9), which has been demonstrated to aid in assembly and secretion of lipid-rich VLDL for delivery to tissues, adipose depots in particular (Legrand and Hermier, 1992).

Plant Systems

Most plants lack desaturases that act on 18:0 other than SACPD. That fact makes this enzyme pivotal in determining the overall amount of fatty acid desaturation in plants. To date, this enzyme, which has been cloned from several species of plants, is the only identified soluble fatty acid desaturase. When Shanklin and Somerville (1991) cloned the *Ricinus communis* (castor) and *Cucumis sativus* (cucumber) SACPDs, they noted 88% amino acid identity between the two enzymes. On the other hand, these soluble desaturases are not genetically similar to the membrane-bound mammalian homologue of SCD. This observation provides strong evidence that the plant and mammalian genes evolved separately.

Expression of cloned plant desaturases in yeast or *E. coli* has provided enough enzyme to conduct enzymatic and crystallographic studies. Both castor and cucumber cDNAs were expressed in yeast and immunoreactive protein was synthesized that had desaturase activity in an *in vitro* assay (Shanklin and Somerville, 1991). Gibson (1993)

verified that palmitoyl-ACP was an alternate substrate for stearoyl-ACP Δ^9-desaturase. Recombinant enzyme expressed in *E. coli* was used to demonstrate that despite its catalytic effect on desaturation, stearoyl-ACP Δ^9-desaturase has a 100-fold greater specificity factor (V_{max}/K_m) for stearoyl-ACP when compared with palmitoyl-ACP. The majority of the difference was because of the difference in V_{max}.

The soluble enzymes that utilize saturated fatty acids bound to ACP as their substrate that have been identified to date include the Δ^9-SACPDs mentioned above, Δ^4-palmitoyl-ACP desaturase of *Coriandrum sativum* (Cahoon et al., 1992), Δ^6-palmitoyl-ACP desaturase of *Thunbergia alata* (Cahoon et al., 1994), Δ^9-myristoyl-ACP desaturase of *Pelargonium xhortorum* (Schultz et al., 1996), and Δ^9-SACPD isolated from milkweed seed. The milkweed seed desaturase has a 10-fold greater specificity for palmitoyl-ACP and a 30-fold greater specificity for myristoyl-ACP than other known Δ^9-desaturases, giving rise to the unique fatty acid composition of milkweed tissue (Cahoon et al., 1997b). This set of enzymes has been shown to have >70% homology, with most of the divergence occurring at the ends of the primary structure. The crystal structure of Δ^9-stearoyl-ACP from *R. cumminus* (Lindqvist et al., 1996) has been very valuable in understanding fatty acid chain length specificity and reaction center mechanisms.

Modifying plants to make fatty acids for special industrial or nutritional applications has been attempted with limited success through traditional plant breeding efforts. More recently, transgenic approaches, as described over 8 years ago by Somerville and Browse (1991), have been employed to shuttle desirable characteristics of oil production from one plant to another plant that is easier to grow or that produces more triacylglycerol. Cahoon et

al. (1997a) have redesigned ACP desaturases to learn about the chain-length and positional specificities of this class of soluble desaturases in plants. In one experiment, Δ^6-palmitoyl-ACP desaturase was mutated by replacing key amino acids from Δ^9-stearoyl ACP desaturase, creating a new series of enzymes capable of desaturating substrates with different number of carbon atoms or at different positions in the acyl chain. With this sort of flexibility, a broad spectrum of lipid products can be envisioned.

Stearoyl-ACP desaturases that have been cloned from plants include safflower (Thompson et al., 1991), cucumber and caster (Shanklin and Somerville, 1991), potato (Taylor et al., 1992), spinach (Nishida et al., 1992), *Brassica napus* (Slocombe et al., 1992), *Brassica rapa* (Knutzon et al., 1992), jojoba (Sato et al., 1992), sesame (Yukawa et al., 1996), and rice (Akagi et al., 1995).

Cahoon et al. (1992) identified a protein from *Coriandrum sativum* (coriander) by using the antibody for Δ^9-SACPD from avocado in an attempt to define the enzyme responsible for petroselinic acid (18:1(n-12)) production in coriander. The antibody reacted with 39- and 36-kDa proteins. Only the 36-kDa protein was in tissues that synthesize 18:1(n-12). The cDNA, isolated by using an expression library, was expressed in tobacco and conferred the ability to produce 18:1(n-12) to the transgenic tobacco callus.

Expression of antisense SACPD message in *Brassica napus* (canola) resulted in dramatic increases (25-fold) in 18:0 concentration in modified plants (Knutzon et al., 1992). Seeds from plants that had the greatest inhibition of SACPD also had a decreased oil content and poor germination. One hypothesis that has been proposed is that, as pools of stearoyl-ACP accumulate, available ACP concentrations drop low enough that fatty acid synthesis is

no longer supported maximally. Seeds that had intermediate activities of SACPD had somewhat normal lipid content and germination rates.

A cDNA isolated from rose petals was found to be homologous to Δ^9 acyl-lipid desaturase from cyanobacteria and acyl-CoA desaturase from mammals (Fukuchi-Mizutani et al., 1995). This is the first known example of sequence homology between desaturases from plants and animals. The putative rose Δ^9-desaturase lacks a leader sequence associated with entry into the chloroplast. The gene is only expressed in mature petals and not in the typical chloroplast-rich tissue, such as leaves. Brown et al. (1991) demonstrated an increased catabolism of PUFA relative to saturated fatty acids in aging carnation petals. This positive correlation between senescence and increased PUFA catabolism had led to the speculation that the senescence-induced desaturase may render fatty acids more susceptible to catabolism.

Coexpression of *A. thaliana* ferredoxin along with palmitoyl-ACP Δ^6-desaturase in *E. coli* results in twice as much 16:1(n-10) as do cells not expressing the exogenous desaturase (Cahoon et al., 1996). Coexpression of exogenous ferredoxin reductase from maize had little effect on desaturase activity, suggesting that *E. coli* are able to maintain a reduced plant ferredoxin or that other substrates such as NADPH of the desaturase electron transport reaction are more limiting.

Yukawa et al. (1996) isolated and studied the expression of two unique SACPDs from sesame. The two genes are expressed differentially in leaf and seed tissue, with one expressing at low levels in leaves and young seeds and the other being unique to seed oil modification. Not only are these gene products useful in the study of plant oil synthesis and

accumulation, but the promoters may be useful in genetic modification of plants when controlled expression of a transgene is desired.

Cahoon et al. (1996) were able to detect 3.2 and 1.4 mol% of 16:1(n-10) and 18:1(n-10), respectively, when they expressed Δ^6-palmitoyl-ACP desaturase from *Thunbergia alata* in *E. coli.* Both acids were increased by 2-fold when ferredoxin from *Arabidopsis thaliana* was coexpressed. Cahoon et al. (1998) have also isolated a cDNA for fatty acyl-ACP desaturase from cat's claw seed (*Doxantha unguis-cati*) by probing with the known Δ^9-SACPD from castor bean. Interest in this species arose when it was noted that up to 80% of the fatty acids in the seed are 16:1(n-7) or 18:1(n-7). The predominant cDNA was very similar to the form from castor bean, but, when expressed in *E. coli*, the enzyme preferred palmitoyl-ACP as a substrate, explaining the unusually large amount of 16:1(n-7) in cat's claw seed. The authors speculate that the substrate preferences between the two desaturases can be explained by a leucine to tryptophan difference at amino acid position 118. This bulky group decreases the depth of the substrate pocket. When the similar mutation was made in Δ^9-SACPD, the specificity for the palmitoyl-ACP substrate was increased to 115% relative to the stearoyl-ACP substrate. Crystallographic data also predict the change in substrate specificity for the Δ^9-SACPD mutant. Learning about natural and engineered differences in desaturases will allow very specific engineering of plants to produce specific oils for industrial, nutritional, or pharmaceutical applications.

Yeast Systems

Yeast have proven to be a good model for studying fatty acid desaturases from plants

or animals. Bossie and Martin (1989) conducted experiments that proved that OLE1, the *Saccharomyces cerevisieae* equivalent of mammalian SCD1, was transcriptionally regulated by supplemented fatty acids in the media. When 18:1(n-9) or 16:1(n-7) were supplemented to the media, the supplemented acid increased in membrane fatty acids, but, because of suppressed Δ^9-desaturase activity, the complementary fatty acid decreased. Supplemented 18:2(n-6) was incorporated into membrane lipids at nearly 50% of the fatty acids, whereas 16:1(n-7) became barely detectable and 18:1(n-9) was decreased by 75%. Northern blotting shows a greater than 80% decrease in mRNA concentration within 60 minutes when yeast are supplemented with 18:2(n-6) or a mix of 18:1(n-9) and 16:1(n-7). The authors suggest the possibility of a soluble factor such as a fatty acid binding protein that may mediate transcriptional control by fatty acids.

The *Saccharomyces cerevisiae* mutant OLE1 is defective in lipid metabolism and requires unsaturated fatty acids for growth. The defective yeast protein in its active state is 57.4 kDa and has an internal region of identity (36%) and similarity (60%) to the rat liver SCD enzyme. When Stukey et al. (1990) expressed the rat SCD in yeast driven by the promoter region and N-terminal 27 codons of *OLE1*, the yeast no longer required unsaturated fatty acids for growth and had similar lipid composition to wild-type control yeast.

Mitchell and Martin (1995) studied the cytochrome b_5-like domain linked to the carboxy terminus of the Δ^9-desaturase in *Saccharomyces cerevisiea*. They noted that disruption of the cytochrome b_5 gene in yeast resulted in viable cells that did not require added sterols or unsaturated fatty acids for growth. When the C-terminal end of the Δ^9-desaturase gene was disrupted in cells that were producing the diffusable cytochrome b_5, the cells became unsaturated fatty acid auxotrophs. When the rat SCD was expressed in

cytochrome b_5 disrupted and OLE1-disrupted yeast, it became obvious that the mammalian form of the enzyme requires cytochrome b_5, but yeast Δ^9-desaturase enzyme has cytochrome b_5 activity.

The effects of fatty acids on OLE1 mRNA stability in yeast as a possible control mechanism involved in fatty acid metabolism was studied by Gonzalez and Martin (1996). OLE1 codes for a membrane-bound protein located on the endoplasmic reticulum that is responsible for Δ^9-desaturation of palmitoyl- and stearoyl-CoA to palmitoleoyl- and oleoyl-CoA. Two independent control systems regulate expression of active enzyme by fatty acid-mediated control of transcription and mRNA stability. Unsaturated fatty acids decrease the message half-life from 10 to less than 2.5 minutes in yeast. Unlike saturated fatty acid activation of transcription, saturated fatty acids have no effect on message half-life. By constructing chimeric constructs of the GAL1 promoter region and the OLE1 5'-UTR and promoter, it was demonstrated that the region of mRNA required for changes in stability related to unsaturated fatty acids is the 5'-UTR of OLE1. Cycloheximide slows nucleolytic degradation of OLE1 mRNA and blocks the unsaturated fatty acid decrease in half-life. Disruption of the XRN1 gene, a 5' to 3' exonuclease, increases the half-life of OLE1 mRNA but does not protect OLE1 mRNA from unsaturated fatty acid destabilization. It is speculated that the two sensing mechanisms that respond from the same stimuli might regulate different pools of fatty acids within the cell.

Messters and Eggink (1996) isolated and characterized a Δ^9-desaturase from *Cryptococcus curvatus* CSB 570, a yeast strain that accumulates up to 60% of its dry weight as triacylglycerol. Cloning of the Δ^9-desaturase from this species is the first step in utilizing this strain of yeast commercially for tailor-made oil production. The newly cloned gene has

62% and 72% homology with the rat and *Saccharomyces cerevisiae* genes, respectively. Oleic acid decreased mRNA concentration for the Δ^9-desaturase by 90%, but 18:1(n-12) did not affect the mRNA concentration.

The promoter region of *Saccharomyces cerevisieae* OLE1 was examined by Choi et al. (1996). A 111-bp, fatty acid-regulated region was found at nucleotide –580. In yeast, this region was critical for the 1.6-fold increase in expression when cells are fed saturated fatty acids and the 60-fold decrease in transcription when unsaturated fatty acids are fed. This region, when inserted into the CYC1 promoter, confers fatty acid responsiveness to CYC1 transcription. Two other genes, fatty acid-activation (FAA)-1 and FAA-4 were found to be required for the unsaturated fatty acid repression of transcription. With one of the FAA genes present, there was an intermediate amount of unsaturated fatty acid responsiveness, but, with both FAA genes deleted, there was no unsaturated fatty acid responsiveness. When the fatty acyl-CoA binding protein gene was missing, transcription increased 5-fold but unsaturated fatty acid repression was not affected.

Gyorfy et al. (1997) expressed the Δ^9-desaturase gene of *Saccharomyces cerevisiea* under the control of other fungal promoters (*H. capsulatum)* and conferred yeast Δ^9-desatuarse activity in a mouse fibroblast cell line. By using this system, active yeast Δ^9-desaturase was expressed in mammalian cells and the MUFA composition of membranes was increased.

Insect Systems

Nutritionally essential fatty acids for animals always have been considered to be 18:2(n-6) and 18:3(n-3). Borgeson et al. (1990) have proposed a Δ^{12}-oleoyl-CoA desaturase

in the American cockroach as demonstrated by efficient conversion of oleoyl-CoA to 18:2(n-6) with little conversion of 18:1(n-9) that is esterified to a phospholipid molecule. To date, this is the only known example of a dietarily essential fatty acid in mammalian diets being synthesized anywhere but in a plants, cyanobacteria, or fungi. Axenic conditions were used to rear house crickets and the American cockroach to eliminate the chance of detecting microbial desaturase activity and to verify that both species had oleoyl desaturase activity (Borgeson et al., 1991). This unique desaturase activity remains controversial, and no specific enzyme or DNA sequence has been identified.

An SCD has been cloned from the salivary gland of the arthropod *Amblyomma americanum* (a species of tick) (Luo et al., 1997). The salivary gland of ticks can increase by about 25-fold in size and protein content during feeding. The mass of 18:1(n-9) increases more than 10-fold during this same time period, indicating the possible crucial role of fatty acid desaturation in supporting this phase of the tick's life cycle. It has also been speculated that SCD is critical in the overwintering process. Now that a clone is available, it will be easier to study this process in more detail.

Wicker-Thomas et al. (1997) identified and sequenced a cDNA from *Drosophila melanogaster* that codes for a protein with homology (up to 40% identical) to mammalian and yeast Δ^9-desaturases. The 383 amino acid protein has two histidine cluster motifs (HXXHH) and two hydrophobic regions. Two forms of the gene have been identified by using the polymerase-chain reaction . One form has three introns, and the other has no introns. *Drosophila melanogaster* is known for having specific hydrocarbon sex pheromones, all with n-7 double bonds but with varying degrees of unsaturation. This gene may be responsible for synthesis of such pheromones as females at different ages express

different concentrations of the mRNA for this newly described gene. Subspecies of *Drosophila melanogaster* produce different arrays of pheromones; therefore, it would be of great interest to learn about the potential differences in the putative desaturase gene. The association of SCD stability and reproductive cycles in the house fly were noted by Wang and Reitz (1983). The half-life of SCD was 9.35 hours in 4-day-old females but only 3.38 hours in 1-day-old females. Evidentely, the half-life increased with age and its expression moved from the integument to the inner body tissue as the female approached ovarian development, indicating a possible increased need for desaturase activity for reproduction.

Fish Systems

Hazel (1984) summarized the lipid altering events that occur when fish are forced to adapt to cold environments. The changes include increased rate of fatty acid desaturation, increased incorporation of fatty acids into phospholipids during fatty acid synthesis, and changes in phospholipid metabolism with the increased rates of desaturation. Wodtke and Cossins (1991) studied cold- induced changes in membrane fluorescence polarization and Δ^9-desaturase activity in carp liver. Upon cooling from 30° C to 10° C, the Δ^9-desaturase activity was increased to a first maximum 3 days after the onset of cooling with a decline in activity back to basal activity with a second and larger maximum at day 10 of cold adaptation, followed by a steep decline to basal levels. Activities in rough and smooth endoplasmic reticulum were assayed separately and had similar responses. The fluorescent polarization, however, increased to about day 3 and then maintained a steady state for the next 7 days.

Tiku et al. (1996) also studied the induction of Δ^9-desaturase in carp liver when the fish were switched to a cold environment. The switch to cold caused a 10-fold increase in both specific activity of the Δ^9-desaturase protein in microsomal membranes and of the mRNA concentration in the cells. These changes took 48-60 hours to occur, but, before this time, there was a modest increase in the specific activity of the Δ^9-desaturase in the hepatic microsomes, probably because of some activation mechanism of latent Δ^9-desaturase. The authors speculated that the mechanism of activation could be myristylation, phosphorylation, or N-glycosylation on the basis of the known sequence of the protein.

Assays

Original methods of assaying desaturase activity utilized ^{14}C-labeled 18:0 or 16:0 as a CoA- or ACP- ester depending on whether one was working with mammalian or plant desaturases. The reaction mixture that contained the appropriate cofactors and buffers was saponified and derivatized after incubation, and 18:0 and 18:1(n-9) or 16:0 and 16:1(n-7) derivatives were separated. Modes of separation included arginated thin-layer chromatography, radio-HPLC, or radio-gas chromatography. Activity of the enzyme preparations then could be determined on the basis of rate of substrate conversion and amount of membrane protein used in the reaction. These methods all relied on multiple analytic steps, many of which are very cumbersome. Thus, the chance for introducing error was great and sample numbers had to be increased to accommodate a "sloppy" assay system.

The use of 9,10-[^{3}H]-18:0 to quantify the activity of SACPD or SCD *in vitro* by measuring the appearance of ^3H_2O simplified the analytical methods greatly (Talamo and Bloch, 1969). Both CoA and ACP-fatty acid thioesters are precipitable by acid after the

reaction has been completed. The precipitate can be collected on a Millipore filter, and the tritiated water extract is counted in a liquid scintillation counter. This method circumvents the need to saponify and separate saturated fatty acids and MUFAs by thin-layer chromatography or other means.

Ivanetich et al. (1996) developed an improved assay system for Δ^6-desaturase activity. The unique ideas that were crucial to the difference from previous systems included separation of reactant and product molecules in the free acid form on an HPLC system. This circumvented the error involved with differential derivatization efficiencies and the use of thin-layer chromatographic plates. The other major change was to account for the free 18:2(n-6) that is associated with the microsomal preparations. By accounting for the microsomal 18:2(n-6), the K_m and V_{max} changed from 1.5 μM and 0.063 nmol/min to 10.7 μM and 0.08 nmol/min.

A simultaneous measure of Δ^5-, Δ^6-, and Δ^9-desaturases has been validated by Su and Brenna (1998) for conducting assays without the use of labeled fatty acids. They compared the results obtained by using stable isotopes followed by gas chromatography/mass spectrometry, and results obtained by using nonlabeled fatty acids and traditional gas chromatography with flame ionization detection. When various tissues with varying concentrations of the three different desaturases were measured, drastic differences by tissue were noted, but both the stable isotope and the non-labeled methods gave the same results. This technique will allow for the study of reactions where labeled substrates are not readily available or where isotope use is not desirable. It is cautioned that this method should be used with systems that are predictable because detection limits might restrict noticing loss of substrate to other unanticipated reactions.

Thia fatty acids can be useful in the study of lipid metabolism because their chemical character is similar to typical fatty acids, but their metabolic fate can be unique, depending on the position of the sulfur atom in the alkyl chain. Skrede et al. (1997) have prepared an extensive review of thia fatty acids and their metabolic effects. Thia fatty acids can be activated to CoA esters, partially β-oxidized, and partially ω-oxidized, resulting in short sulfoxy dicarboxylic acids. Some isomers can be desaturated and incorporated in phospholipids, often times causing changes in (n-3) to (n-6) ratios. Three-thia fatty acids induce PPAR-α and the retinoid-X receptor-α, inhibit cholesterol and malonyl-CoA synthesis, and activate the enzymes of β-oxidation, leading to a hypolipidemic effect. Four-thia fatty acids can inhibit β-oxidation, leading to fatty liver, and 9-thia 18:0 is a strong inhibitor of Δ^9-desaturase. Apart from research uses, the thia fatty acids hold the greatest hope of being hypolipidemic agents used in place of the fibrates, which have more negative side-effects.

Frøyland et al. (1997) utilized three-thia fatty acids, known mitochondrial and peroxisome proliferators, to better understand factors affecting fatty acid composition and lipoprotein metabolism. C12-S-Acetic acid and C14-S-acetic acid were the most hypolipidemic, decreasing plasma triacylglycerols by 50%. At the same time, Δ^9-desaturase gene expression was increased resulting in a greater concentration of hepatic 18:1(n-9). Oxidation of 20:5(n-3) increased, probably because of increased peroxisomal enzymes and decreased plasma VLDL and PUFAs (20:5(n-3)), leading to a less oxidatively atherogenic lipoprotein profile.

Miscellaneous Related Information

Broido et al. (1991) studied the expression of *Lemma gibba* light harvesting complex IIb AB30 or AB19 in COS-7 cells to understand cellular targeting of chloroplast-destined proteins in an animal cell. The cDNAs were expressed under the control of an SV40 promoter but were not processed and accumulated in structures resembling inclusion bodies within the COS-7 cells.

After 18.5 days of space flight on the Soviet Cosmos 936 biosatellite and after 25 days of recovery after flight, 28 hepatic enzymes were assayed in rats (Abraham et al., 1983). Five enzymes decreased in activity including glycogen phosphorylase, 6-phosphogluconate dehydrogenase, aconitate hydratase, α-glycerolphosphate acyltransferase, and diglycerol acyltransferase because of space flight. Two enzymes including palmitoyl-CoA desaturase and lactate dehydrogenase increased during flight. Both liver glycogen and the ratio of 16:0 to 16:1(n-7) increased, supporting the *in vitro* assays for glycogen phosphorylase and palmitoyl-CoA desaturase. All seven enzymes that changed because of flight had returned to normal after the 25-day recovery period. This study reinforces the concern of long-term weightlessness on humans in space stations.

Albert and Coniglio (1977) presented evidence for what they considered Δ^8-desaturase activity in rat testes. By injecting radioactive $20{:}2\Delta^{11,14}$ and sampling tissue for radioactive fatty acid composition 8 hours later, they found 51.5% of the label as the original compound. The metabolite with the most radioactivity was 20:3(n-6) which contained 29.5% of the counts. Ozonolysis of the 20:3(n-6) acids resulted in about 85% as the 5-carbon aldehydo ester (indicating $20{:}3\Delta^{5,11,14}$) and 15% as the 8-carbon aldehydo ester (indicating

20:3$\Delta^{8,11,14}$). Microsomes from testes resulted in similar *in vitro* results, whereas hepatic microsomes did not exhibit Δ^8-desaturase activity

Cadena et al. (1997) cloned a member of the membrane lipid desaturase (MLD) family by using the yeast two-hybrid system with a fragment of the epidermal growth factor receptor as the binding protein. When expressed, the MLD has three consensus motifs, HX_3H, HX_2HH, and HX_2HHXFP that fit the character of other fatty acid desaturases. The conserved histidine residues are proposed by Shanklin et al. (1994) to be crucial in binding the catalytic iron. Expression of this membrane-bound protein is quite ubiquitous, occurring in all 16 of the human tissues tested. Other than the three conserved histidine regions, the protein does not share homology with other known fatty acid desaturase genes. Overexpression of MLD specifically inhibits epidermal growth factor receptor expression. The authors speculate that is possible that MLD expression could coordinate fatty acid metabolism with regulation of the epidermal growth factor receptor.

It has been accepted that cats do not have the ability to perform Δ^6-desaturation, as they became "essential fatty acid deficient" and had low levels of 20:4(n-6) when fed safflower oil as their fat source (Rivers et al., 1975). Pawlosky et al. (1994) utilized sensitive gas chromatography-mass spectrometry methods to identify Δ^6-desaturation products in cats fed 9:1 hydrogenated coconut oil:corn oil. After a 6-month feeding period, deuterium labeled 18:3(n-3) and 18:2(n-6) were given orally and tissues were collected for analysis. Labeled 18:3(n-6), 22:4(n-6), and 22:5(n-3) were found in the plasma, indicating hepatic Δ^5- and Δ^6-desaturase activities, whereas 22:6(n-3), 24:5(n-3), 24:6(n-3), 22:5(n-6), 24:4(n-6),

and 24:5(n-6) were found only in brain, indicating that neural tissue is unique in its ability to perform Δ^4-desaturation.

Lee et al. (1998b) have identified and cloned a cDNA from *Crepis alpina* encoding an enzyme capable of forming acetylenic (triple) bonds in fatty acids. Formation of crepenynic acid (9-octadecen-12-ynoic acid) from 18:2(n-6) was detected. Up to 25% of the total fatty acids were determined to be crepenynic acid in transgenic *Arabidopsis* expressing the newly cloned acetylenase. The primary sequence of the acetylenase indicates that it is likely to be a non-heme diiron protein such as the fatty acid desaturases, hydroxylases, and epoxigenases found in plants, animals, fungi, and bacteria. Because these enzymes all contain histidine-rich motifs, and are capable of stabilizing a diiron center where oxidation of the substrate occurs, they are classified together. The economic potential of transferring unique fatty acid processing capabilities to easy-to-grow oil crops are great and the reality of this scenario is fast approaching.

Pugh and Kates (1979) demonstrated a functional phospholipid desaturase in rat liver by using a purification scheme for SCD published by Strittmatter et al. (1974). These enzyme preparations had a 7-fold increase in activity compared with crude microsomes. The enzyme converted 2-eicosatrienoyl-lecithin to 2-arachidonoyl-lecithin without a lag period and in a linear fashion with added protein or increased time. They minimize the possibility that phospholipase A_2 could release free fatty acids that then would be activated by a thiokinase to their CoA derivative, which then could be desaturated by the traditional fatty acid CoA desaturase and re-esterified to the lecithin. They argue that there was no release of free fatty acid and that ATP or CoA, required compontents in reacylation, were not necessary in the reaction mixture.

Schenck et al. (1996) have proposed Δ^8-desaturase activity in mouse liver. By dosing mice with 20:3(n-3) with deuterium at 3,3,4,4,8,8,9,9 carbon positions, 4.4% of the deuterium products proceeded through a Δ^8-desaturase pathway. The majority of the deuterated products (35.3%) were retroconverted to 18:3(n-3)-d_6 and then elongated and desaturated via typical Δ^5- and Δ^6-desaturases and elongases. Greater than 22% remained as the original deuterated fatty acid, and 28.9% were typical elongation and desaturation products of the original deuterated fatty acid. To detect Δ^8-desaturase activity, the mice were fed marginally EFAD-diets. The deuterium label may cause an isotope effect by inhibiting the Δ^6-desaturase and increasing the chance of Δ^8-desaturation.

Schultz et al. (1996) identified the gene for a unique fatty acid desaturase, Δ^9-myristoyl-ACP desaturase. The (n-5) product is a necessary precursor to 16:1(n-5) and 18:1(n-5) which ultimately are needed for anacardic acid synthesis, a secondary compound derived from fatty acids. Anacardic acid often is associated with pest resistance in plants. The gene was cloned from pest-resistant geraniums (*Pelargonium xhortorum)* and was not found to be expressed in pest-susceptible geraniums.

Spychalla et al. (1997) searched the *Caenorhabditis elegans* DNA database to compare known sequences from *Arabidopsis*. Several putative desaturases were identified on the basis of sequence homology. One of the genes identified, *fat-1,* had greater than 30% homology with FAD2 (Δ^{12}-desaturase) and FAD3 (n-3 desaturase). The unique nature of *fat-1* was discovered when it was expressed in *A. thaliana* and caused a 90% increase in root lipid 18:3(n-3), as well as converting 20:3(n-6) to 20:4(n-3) and 20:4(n-6) to 20:5(n-3).

Thus, the *fat1* gene is the first example of an animal glycerolipid desaturase, capable of converting (n-6) acids to (n-3) acids, previously only identified in plants and cyanobacteria.

Ves Losada and Brenner (1995) have demonstrated Δ^5-desaturase activity in rat liver nuclei, the first timemammalian fatty acid desaturase activity has been documented in membranes other than endoplasmic reticulum. The nuclear enzyme had a pH optimum of 7.6 and required the yet-to-be identified cytosolic factor that microsomal Δ^5-desaturase requires for full activity. Long-chain fatty acyl-CoA synthetase activity also was found in nuclear membranes. While the nuclear membrane is linked directly to the endoplasmic reticulum, it is not with great surprise that other endoplasmic reticulum proteins such as cytochrome b_5 and cytochrome P_{450} are also found in nuclei at low specific activities. It is speculated, however, that minor changes in fatty acid composition of nuclear membranes could exert large changes in cell metabolism by changing passage of mRNA or nucleoside triphosphates through the nuclear membrane.

Tebbey and Buttke (1992a) demonstrated that β-lymphocytes from BALB/c (normal) and MRL/lpr (autoimmune strain) mice express SCD2, as opposed to SCD1, which is expressed in liver exclusively. BALB/c T cells do not express either form of SCD, but BALB/c thymocytes and MRL/lpr T cells express SCD2, demonstrating a developmental down-regulation of SCD2, which also is known to occur in the presence of 20:4(n-6). SCD1 was not detected in any lymphoid cells.

Summary

Genetic, dietary, environmental, hormonal, pharmaceutical, and other unknown factors control the actions of fatty acid desaturases. The complexity of desaturase control is

deserving for such a vital system. The maintenance of all biological processes, in some way depends on the integrity of membrane structure, which, is in part determined by the availability of an optimal ratio of fatty acids. Other crucial molecules such as prostaglandins and leukotrienes also originate from the fatty acid desaturase system. To obtain a better overview of the myriad of controlling factors of the fatty acid desaturase systems examined in this review, the following tables help summarize the data in the literature. Tables 1, 2, 3, and 4 compile the agents used and the effects observed on Δ^9-desaturase *in vivo,* Δ^9-desaturase *in vitro,* Δ^6-desaturase, and Δ^5-desaturase, respectively. Figures 3 and 4 condense the observations in the literature into a summary of inhibitors and activators of the Δ^9-desaturase system and the Δ^5- and Δ^6-desaturase systems, respectively.

Table 1. Summary of Δ^9-desaturase *in vivo* modulators.

Agent	Effect	Reference
β-Carotene or 13-*cis*-retinoic acid fed to rats	Decreases activity with above normal doses	Alam et al., 1984
(n-3) Fatty acids fed to mice	Decrease SCD activity via insulin or insulin receptor interuption in liver	de Antueno et al., 1993
11-Deoxycorticosterone injections given to rats	Increases acitivity	Marra and de Alaniz, 1991
17-β-Estradiol injections given to roosters	Increases activity	Lipiello et al., 1979
4% Corn oil fed to pigs	Decreases SCD activity in adipose tissue	Kouba and Mourot, 1998
9-*cis*,12-*cis* Configuration PUFAs fed to rats	Maximal inhibition in liver compared with isomers having *trans* bonds	de Alaniz et al., 1986
Androgen injections given to rats	Decreases activity	Thorling and Hansen, 1995
Branched-chain fatty acids fed to rats	Decreases hepatic SCD activity in liver	Wahle and Hare, 1980
Cadmium fed to rats	Inhibits activity by antagonizing zinc	Kudo et al., 1991
Casein fed to rats	Decreases activity when 45% vs 5% is fed	Peluffo et al., 1984
Cholesterol fed to rats	Increases SCD activity	Garg et al., 1988a; Muriana et al., 1992; Garg et al., 1986;Leikin and Brenner, 1987; Landau et al., 1997;
Clofibric acid fed to mice	Increases activity	Kawashima et al., 1986
Clofibric acid fed to mice	Incerases transcription	Miller and Ntambi, 1996
Clofibric acid fed to rats	Increases activity	Kawashima and Kozuka, 1982; Kawashima et al., 1986; Kawashima et al., 1985;
Cold environment for fish	Increases activity	Tiku et al., 1996
Cold environment for male rats	Decreases activity (no difference when pair-fed)	Ves Losada and Peluffo, 1987
Cold environments for femle rats	Decreases activity	Gonzalez et al., 1983
Cold temperature treatment of tetrahymena	Increases activity	Kasai and Nozawa, 1980
Conjucated linoleic acid fed to mice	Decreases SCD activity in liver	Lee et al., 1998
Corn oil/coconut oil fed to rats	Increases/decreases activity in liver	Pugh and Kates, 1984
Dexamethasone injections given to rats	Increases activity	Marra et al., 1988
Diabetic rats	Decreases activity	Kawashima et al., 1985; Mimouni et al., 1992; Prasad and Joshi, 1979; Clark and Queener, 1985
Estrogen injections given to rats	Increases activity	Thorling and Hansen, 1995
Ethanol fed to rats	Decreases activity	Umeki et al., 1984; Rao et al., 1984

Table 1. (continued)

Agent	Effect	Reference
Fasting and refeeding high carbohydrate diet to rats	Increases activity	Thiede and Strittmatter, 1985
Fish oil fed to rats	Increases SCD activity relative to olive oil	Muriana et al., 1992
Fish oil or lindseed oil fed to rats	Decreases SCD activity	Garg et al., 1988a
Fructose fed to diabetic mice	Increases SCD activity	Waters and Ntambi, 1996
Fructose fed to streptozotocin treated rats	Increases activity	Prasad and Joshi, 1979
Glucagon injections given to mice	Decreases expression	Waters and Ntambi, 1994
Growth hormone injections given to rats	Decreases activity	Gueraud and Paris, 1997
Hot environments for yeast	Increases activity	Guerzoini et al., 1997
Hybernation of hamsters	Increases brain MUFA via liver not brain SCD	Goldman, 1978
Hyperglycemic and diabetic rats	Decreases activity	Mimouni and Poisson, 1992
Insulin administration and fat-free feeding of rats	50-fold increase in specific activity in liver	Oshino and Sato, 1972
Insulin injections given to diabetic mice	Increases expression	Waters and Ntabmi, 1994
Insulin injections given to diabetic rats	Increases activity	Faas and Carter, 1980; Kawashima et al., 1985; Nishida et al., 1988; Mimouni et al., 1992; Prasad and Joshi, 1979
Iron deficient diet fed to rats	Decreases activity	Rao et al., 1983
Low protein diet fed to rats	Decreases activity	Narce et al., 1992
Low protein/essential fatty acid deficient diets fed to nursing rat dams	Decreases (60%)/increases (360%) activity in pups	De Tomas et al., 1980
Margarine fed to rats	Decreases activity relative to hydrogenated coconut oil	Mahfouz et al., 1981
Medium-chain triacylglycerols fed with corn or olive oils to rats	Decreases SCD activity in liver	Periago et al., 1989
ob/ob Mice	Increase in activity because of increased caloric intake	Enser and Roberts, 1982
Obese Zucker rats	Increases activity	Jones et al., 1996
Partially hydrogenated marine oils fed to rats	Decrease hepatic activity compared with hydrogenated peanut oil	Svensson, 1983
Perfluoro-octanoic acid fed to mice	Increases acitivity in males but not females	Kawashima et al., 1989
Refeeding after starvation	Increases activity	Kawashima et al., 1985
Retinol palmitate fed to mice	Increases expression	Miller et al., 1997
Starvation of rats	Decreases activity	Kawashima et al., 1985
Testosterone injections given to male or female rats	Increases activity	Marra and de Alaniz, 1989
Thyroxine injections given to rats	Incresaes activity	Joshi and Aranda, 1979; Nishida et al., 1988

Table 1. (continued)

Agent	Effect	Reference
Triiodothyronine injections given to rats	Increases activity	Ves Losada and Peluffo, 1989; Nishida et al., 1988
Vitamin A deficient diets fed to rats	Increase in activity	Alam and Alam, 1985
Zinc deficient diets fed to rats	Decreases activity	Eder and Kirchgessner, 1995

Table 2. Summary of Δ^9-desaturase *in vitro* modulators.

Agent	Effect	Reference
β-Estradiol given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
12 kDa Rat liver cytosolic protein in *in vitro* reaction	Increase activity by preferentially binding 18:1 vs 18:0	Catalá, 1986
26.5 kDa Rat liver cytosolic protein in *in vitro* reaction	Increase activity by organizing multienzyme complex	Jones and Gaylor, 1979
Aldosterone given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
Arachidonic acid given to 3T3-L1 cells in culture	Decreases activity and mRNA	Sessler et al., 1996
Bovine serum albumin and rat liver cytoplasm in *in vitro* reaction	Increase activity by protection from thioesterases	Jeffcoat et al., 1977
Cholesterol added to hepatic microsomes *in vitro*	Increases activity	Garda and Brenner, 1985
Corticosterone given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
Cortisone given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
Cu^{++} ions in *in vitro* reactions	Decrease activity	Sreekrishna and Joshi, 1980
Deoxy corticosterone given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
Dexamethasone given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
Differentiation of 3T3-L1 cells in culture	6-Fold increase in activity	Kasuri and Joshi, 1982
Ethanol given to HepG2 cells in culture	Decreases activity	Angeletti and de Alaniz, 1996
Insulin given to differentiated 3T3-L1 cells in culture	100-Fold increase in activity	Kasuri and Joshi, 1982
Insulin/PUFA given to H2.35 cells in culture	Increases/decreases activity but PUFA over rides insulin	Ntambi et al., 1996
Methylisobutylxanthine given to 3T3-L1 cells in culture	Increases expression	Casimir and Ntambi, 1996
Retinoic acid given to 3T3-L1 cells in culture	Decreases activity	Stone and Bernlohr, 1990
Testosterone given to rat hepatocytes in culture	Increases activity	Marra and de Alaniz, 1995
Thiazolidinediones given to 3T3-L1 cells in culture	Decreases expression	Kurebayashi et al., 1997
TNF-α given to 3T3-L1 cells in culture	Decreases expression	Weiner et al., 1991

Table 3. Summary of Δ^6-desaturase modulators.

Agent	Effect	Reference
11-Deoxycorticosterone injections given to rats	Decreases activity	Marra and de Alaniz, 1990
13-*cis*-Retinoic acid fed to rats	Increases activity	Alam et al., 1984
18:2(9 *trans*,12 *trans*) Given to human Jurkat-T cells in culture	Inhibits 18:3(n-3) conversion to 22:6(n-3) more strongly than from 20:5(n-3)	Marzo et al., 1996
Adrenocorticotropic hormone injection given to rats	Decreases activity	Mandon et al., 1987
Aging in humans	Decreases activity based on fatty acid composition	Raederstorff et al., 1994
Aging in mice	Decrease in liver activity	Bourre et al., 1990
Aging in rats	Decreases activity	Bordoni et al., 1988
Aging in rats	Increases activity on 18:2(n-6)	Maniongui et al., 1993
Aging in rats	Decreases activity in heart microsomes	Jimenez et al., 1993
Aldosterone injections given to rats	Decreases activity	Marra and de Alaniz, 1990
Casein fed to rats	Increases activity when 45% vs 5% is fed	Peluffo et al., 1984
Cholesterol added to hepatic microsomes *in vitro*	Increases activity	Garda and Brenner, 1985
Cholesterol fed to rats	Decreases activity when fed with tallow	Garg et al., 1988c
Cholesterol fed to rats	Decreases activity	Garg et al., 1986; Osada et al., 1995; Leikin and Brenner, 1987; Muriana et al., 1992
Cholesterol fed to rats with soy or casein protein	Decreases activity	Lindholm et al., 1993
Cold environment for femle rats	Increases activity	Gonzalez et al., 1983
Corn oil/coconut oil fed to rats	Increases/decreases activity in liver	Pugh and Kates, 1984
CP-24979 Fed to mice	Decreases activity	Obukowicz et al. 1998
Dexamethasone injections given to rats	Decreases activity	Marra et al., 1986a
DL-α-Tocopherol added in 20-fold excess to microsomes *in vitro*	Decreases activity from liver microsomes, increases activity from brain microsomes	Despret et al., 1992
Epinephrine injections given to rats	Decreases activity	Mills et al., 1995
Eritadenine added to microsomes in *in vitro* assays	Decreases activity	Sugiyama et al., 1997
Essential fatty acid deficient media given to HepG2 cells in culture	Increases activity	Melin and Nilsson, 1997
Ethanol fed to Yucatan barrow micropigs	Decreases activity	Nakamura et al., 1994
Fish oil fed to rats	Decreases activity compared with olive oil	Muriana et al., 1992

Table 3. (continued)

Agent	Effect	Reference
Fish oil fed to rats	Deccreased activity	Dihn et al., 1995
Fish oil or linseed oil fed to rats	Decreases activity compared with tallow	Garg et al., 1988c
Gamma-linolenic acid fed to aged rats	Increases activity especially of (n-3) metabolites	Biagi et al., 1991
Glucagon injections given to rats	Decreases activity	Gomez-Dumm et al., 1975
Growth hormone expression in transgenic mice	Increases activity in liver and adipose	Nakamura et al., 1996
Hyperglycemic and diabetic rats	Decreases activity	Mimouni and Poisson, 1992
Large doses of tyrosine or phenylalanine fed to rats	Decrease activity via increased epinephrine and cAMP	Peluffo et al., 1984
Lipoprotein-like cytosolic factor from rat liver in *in vitro* reactions	Increases activity by sequestering 18:3(n-6)	Leikin, and Brenner, 1986
Low protein diet fed to nursing rat dams	Decreases (60%) activity in pups	De Tomas et al., 1980
Low protein diet fed to rats	Decreases activity	Narce et al., 1988
Margarine fed to rats	Decreases activity relative to hydrogenated coconut oil	Mahfouz et al., 1981
Medium-chain triacylglycerols fed with corn oil to rats	Decreases activity in liver	Periago et al., 1989
Obese children	Increases activity via incrased fasting insulin	Decsi et al., 1996
Partially hydrogenated marine oils fed to rats	Decrease activity compared with hydrogenated peanut oil	Svensson, 1983; Kirstein et al., 1983
Soy protein fed to rats	Decreases activity relative to casein fed rats	Madani et al., 1998; Lindholm et al.; 1993, Koba et al., 1993
Spontaneous hypertensive rats	Decreases activity	de Catalfo and de Gómez-Dumm, 1996
Streptozotocin treated rats	Increases activity in kidney	Clark and Queener, 1985
trans Fatty acids fed to rats	Decreases activity	De Schrijver and Privett, 1981; Mahvouz et al., 1984
Triiodothryonine injections given to humans	Decreases activity based on plasma fatty acids, but increases activity based on erythrocyte and polymorphonuclear leukocyte fatty acid composition	van Doormaal et al., 1986
Triiodothyronine injections given to rats	Decreases activity	Ves Losada and Peluffo, 1989

Table 4. Summary of Δ^5-desaturase modulators.

Agent	Effect	Reference
(+)-Sesamin in *in vitro* assays	Decreases activity	Shimizu et al., 1991
Adrenocorticotropic hormone injections given to rats	Decreases activity	Mandon et al., 1987
Casein fed to rats	Increases activity when 45% vs 5% was fed	Peluffo et al., 1984
Cholesterol added to hepatic microsomes *in vitro*	Increases activity	Garda and Brenner, 1985
Cholesterol fed to rats	Decreases activity	Garg et al., 1986; Leikin and Brenner, 1987
Cholesterol fed to rats with fish oil/olive oil	Increases/decreases activity	Muriana et al., 1992
Cholesterol fed to rats with soy or casein protein	Decreases activity	Lindholm et al., 1993
Cholesterol fed with fish oil or linseed oil to rats	Decreases activity	Garg et al., 1988b
Choline deficient diets fed to rats	Decreases activity	Leikin and Brenner, 1992
Cold environments for femle rats	Increases activity	Gonzalez et al., 1983
Corn oil/coconut oil fed to rats	Increases/decreases activity in liver	Pugh and Kates, 1984
CP-24979 Fed to mice	Decreases activity	Obukowicz et al. 1998
Dexamethasone injections given to rats	Decreases activity	Marra et al., 1986a
Diabetic rats	Decreases activity that was increased with insulin	Shin et al., 1995
Epinephrine administered to rats	Decreases activity	Mills et al., 1995
Essential fatty acid deficient media given to HepG2 cells in culture	Increases activity	Melin and Nilsson, 1997
Ethanol fed to Yucatan barrow micropigs	Decreases activity	Nakamura et al., 1994
Ethanol given to HepG2 cells in culture	Decreases activity	Angeletti and de Alaniz, 1996
fa/fa Rats	Decreases activity	Blond et al., 1989
Fat free diet/(n-6) fatty acids fed to rats	Decrease/increase activity in liver	Gomez-Dumm et al., 1983
Fish oil fed to rats	Decreases activity compared with olive oil	Muriana et al., 1992
Fish oil/linseed oil fed to rats	Decreases/increases activity relative to tallow feeding	Garg et al., 1988b
Growth hormone injections given to rats	Decreases activity	Gueraud and Paris, 1997
Hyperglycemic and diabetic rats	Decreases activity	Mimouni and Poisson, 1992
Insulin injections given to humans	Increases activity based on fatty acid composition of plasma	Boustani et al., 1989
Lipoprotein-like cytosolic factor from rat liver in *in vitro* reactions	Increases activity by sequestering 20:4(n-6)	Leikin, and Brenner, 1989

Table 4. (continued)

Agent	Effect	Reference
Low protein diet fed to nursing rat dams	Decreases (60%) activity in pups	De Tomas et al., 1980
Low protein diet fed to rats	Decreases activity	Narce et al., 1988
Partially hydrogenated marine oils fed to rats	Decrease activity compared with hydrogenated peanut oil	Svensson, 1983; Kirstein et al., 1983
Saturated fatty acid fed to rats	Decreases activity	Dang et al., 1985
Spontaneous hypertensive rats	Decreases activity	de Catalfo and de Gómez-Dumm, 1996
trans (n-9) Fatty acids given to human fibroblasts in culture	Inhibits activity whereas *trans* (n-7) fatty acids do not	Rosenthal and Doloresco, 1984
Triiodothyronine injections given to rats	Decreases activity	Ves Losada and Peluffo, 1989

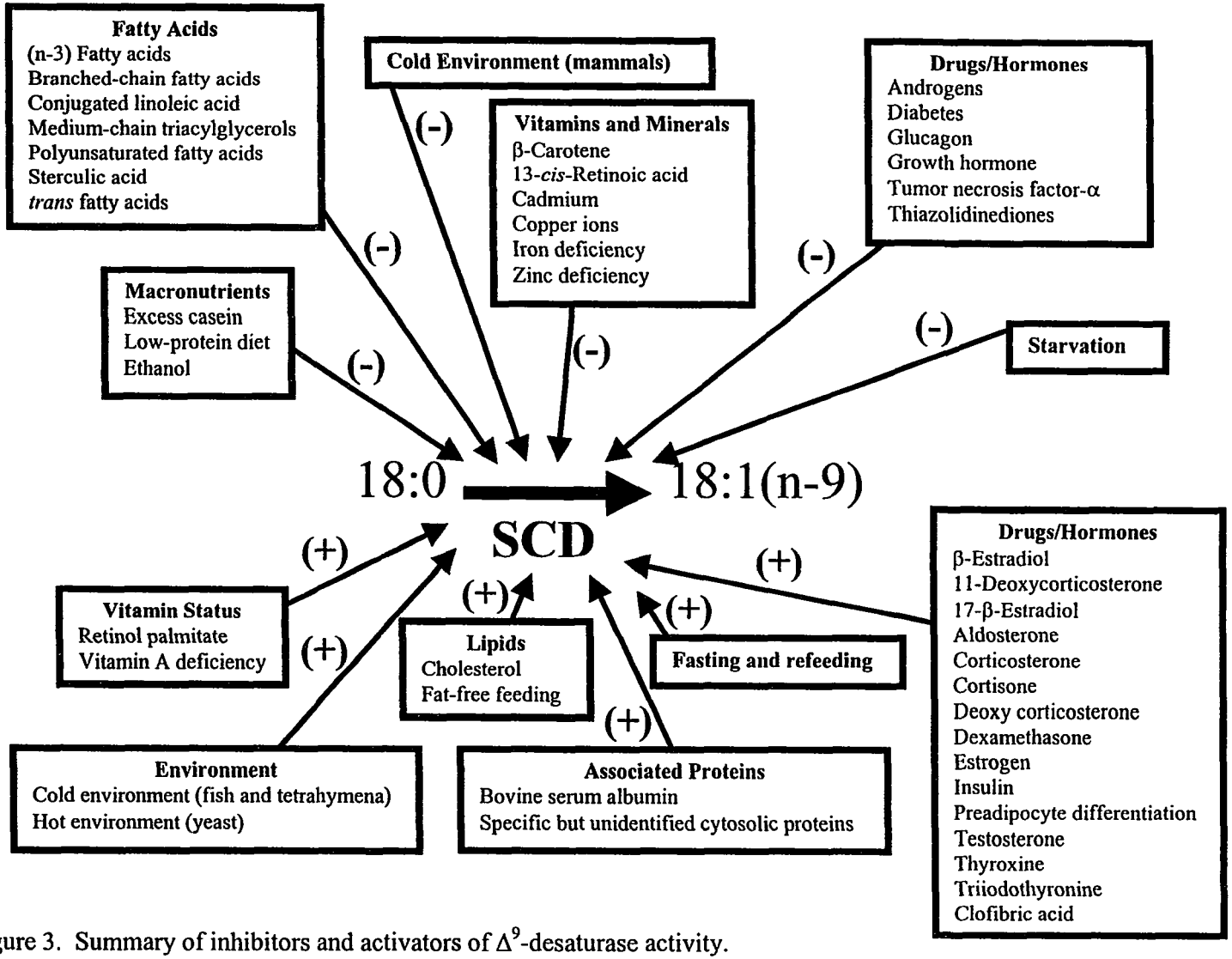


Figure 3. Summary of inhibitors and activators of Δ^9-desaturase activity.

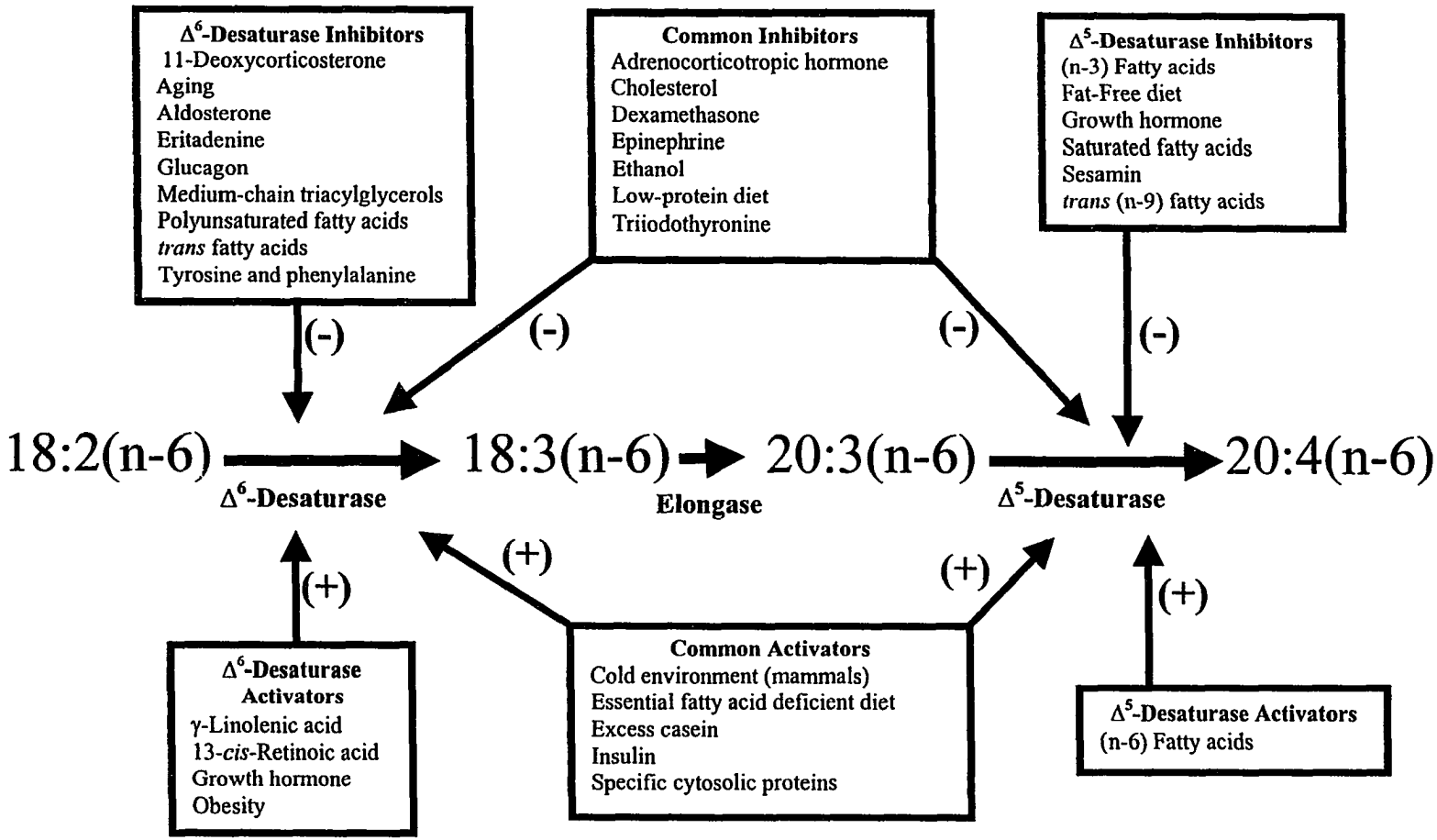


Figure 4. Summary of inhibitors and activators of Δ^5- and Δ^6-desaturases.

INCREASE IN MONOUNSATURATED FATTY ACIDS IN COS-1 CELLS BY EXPRESSION OF EXOGENOUS MAMMALIAN DESATURASE

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Abstract

COS-1 cells were transfected transiently with expression vectors encoding stearoyl-acyl-carrier-protein desaturase (SACPD) from castor bean, stearoyl-CoA desaturase (SCD) from mice, and a control vector containing SACPD cDNA in reverse orientation. The hypothesis was that both exogenous desaturases would be active in COS-1 cells, thereby increasing the proportion of monounsaturated fatty acids produced by the cells. Messenger RNA and anti-myc immunoreactive protein was detected at 12 and 24 hours post-transfection respectively and increased through 48 hours post-transfection. When acetate was used as the fatty acid precursor in cultures for a 24-hour period starting 24 hours post-transfection, the ratio of monounsaturated to saturated fatty acids was increased in the cells expressing SCD ($P<.05$) relative to SACPD-expressing cells and control cells, which did not differ from each other. Increased proportions of specific monounsaturated acids were synthesized in cells expressing SCD ($P<.05$), relative to SACPD-expressing and control cells. Control cells were numerically intermediate in the production of monounsaturated fatty acids compared with SCD- and SACPD-expressing cells. The presence of additional SCD increases the fatty acid

desaturase capacity of cells, whereas SACPD is not active in mammalian cells. From this research, it is conceivable to think seriously about tailoring the fatty acid composition of food products derived from animals by controlling the rates of mammalian desaturation by modulating transcription of endogenous or adding exogenous desaturases.

Introduction

Animal products clearly are an important part of the Western diet. Data from the Eating in America Today edition II (EAT II) study, conducted by the National Livestock and Meat Board (National Livestock and Meat Board, A Dietary Pattern and Intake Report, 1994), indicate that 55% of dietary fat comes from the meat and milk food groups. Similarly, 64% of dietary saturated fatty acids and 70% of dietary protein comes from these animal sources. Many people, however, have questioned the wisdom of consumption of animal products with regards to risk of heart and vascular disease. When asked to identify their major health concern about the healthfulness of the American diet, leading nutritionists most frequently criticize the amount of saturated fatty acids consumed daily. In a symposium, the Wisconsin Milk Marketing Board suggested the ideal nutritional fatty acid composition for milk fat would contain 82% monounsaturated fatty acids rather than 25%, with the increase occurring at the expense of saturated fatty acids (O'Donnell, 1989). Changes of similar magnitude also would be appropriate for the fatty acid composition of meat products. This viewpoint is strengthened by the Surgeon General's Report on Nutrition and Health in which the number one recommendation is that most people should decrease consumption of fat (especially saturated fat) and cholesterol (U.S. Department of Health and Human Services, 1988).

As the effects of specific fatty acids on disease conditions are determined, the need to modify the fatty acid composition of the human diet will increase. Many examples of

modifying fatty acid composition are already evident in plant-derived lipids such as high-18:0 soybean oil (Hartmann et al., 1997) and low erucic acid canola oil (Lammerink and Morice, 1971). Attempts to change the fatty acid composition of animal fats, however, have not been as successful on a large scale. The major sources of animal fat in the human diet are beef, pork, and milk fat, which all contain greater than 50% saturated fatty acids. Changing fatty acid composition of muscle tissue of monogastric animals is relatively easy compared with that in ruminants because dietary fatty acids, unlike those in ruminants, are absorbed and used in membrane synthesis and for energy storage in the same form that they exist in the diet. There are many examples where dietary fats have been fed to pigs to change the fatty acid composition of pork fat (Skelley et al., 1975; Wood, 1984; and Miller et al., 1990). In ruminants, however, dietary unsaturated fatty acids are saturated in the highly reductive rumen before absorption (Ashes et al., 1997). This phenomenon leaves fatty acid desaturase systems of ruminants in greater control of milk and meat fatty acid composition.

Gibson's 1991 review summarizes the potential means for changing fatty acid composition in milk fat. There are modest differences of milk fatty acid composition between and within breeds of dairy cattle, indicating that traditional breeding practices could use selection criteria to develop genetic strains to produce tailor-made fatty acids. He indicates that another approach would be to use the tools of genetic engineering to manipulate the genes responsible for fatty acid synthesis and modification. Despite the probable chances of success in significantly modifying the fatty acids in milk via traditional or modern methods, the likelihood of designing specific strains of cows for specific niche markets is unlikely for economic reasons.

There are three types of fatty acid desaturases: acyl-CoA desaturases, acyl-ACP desaturases, and acyl-lipid desaturases (Murata and Wada, 1995). Acyl-CoA desaturases are located in the endoplasmic reticulor membrane of animal cells, yeast, and other fungal cells (Holloway, 1983). Acyl-ACP desaturases are located in the stroma of plant tissue (McKeon and Stumpf, 1982). Acyl-lipid desaturases are bound to endoplasmic reticular and chloroplast membranes of plant cells (Schmidt and Heinz, 1993) and the thylakoid membranes of cyanobacterial cells (Wada et al., 1993).

To learn about expression of foreign desaturases in mammalian cells, COS-1 cells were chosen as a cell line that expresses high levels of recombinant proteins (Tran-Paterson et al., 1990). The SACPD from castor bean was selected as an example of a non-membrane bound Δ^9-desaturase and SCD from mouse as an example of a typical membrane-bound mammalian Δ^9-desaturase. The goal was to increase the total Δ^9-desaturase capacity and hence incease 18:1(n-9) and 16:1(n-7) of the cell by targeting the cytosolic site of fatty acid synthesis with fatty acid desaturation via SACPD and the endoplasmic reticulum site of fatty acid desaturation via excess SCD.

Materials and Methods

Vector Preparation

Polymerase chain reaction (PCR) using primers with 5' overhangs (FIG. 1A) was used to amplify and modify the ends of the cDNA for SCD from mouse (Ntambi, 1988) and the cDNA for stearoyl-acyl carrier protein desaturase (SACPD) from castor bean (Shanklin and Somerville, 1991). In the cases of SACPD, the 5' Kozak sequence was modified to the consensus mammalian Kozak sequence (...CAACC**ATG**G...Kozak, 1991) and the 32 amino acid leader sequence that is thought to target the protein to the chloroplast or plastid in plants

was not included in the expression vector.In the case of SCD, the long 3' untranslated region was not included in the expression vector. The PCR products were digested with restriction enzymes Nhe I and Xho I and ligated into the mammalian expression vector pcDNA3.1(+)zeo (Invitrogen, Carlsbad, CA) that had been modified by inserting paired oligonucleotides (FIG. 1A) encoding the myc epitope into the Xho I and Xba I restriction sites. The final products were SACPD and SCD cDNAs fused in frame with C-terminal myc epitopes in the multiple cloning site of pcDNA3.1(+)zeo (FIG. 1B and 1C). The control plasmid consisted of the Eco RI coding region for SACPD in the reverse orientation in the Eco RI restriction site of pcDNA3.1(+)zeo. Host *E coli.*, XL-1 blue, were used to amplify plasmids in the presence of 25 μg/mL zeocin (Invitrogen, Carlsbad, CA) in Luria-Bertani media grown in a shaking flask at 37° C. Qiagen Maxi-500 columns (Qiagen, Chatsworth, CA) were used to purify high quality supercoiled plasmids. After purification and before transfection, the plasmids were screened with restriction enzymes, the coding region was sequenced, and *in vitro* transcription and translation (TnT Kit, Promega Corp., Madison, WI) was used to assure production of a product with the predicted molecular weight.

Cell culture and Transient Transfection

COS-1 cells were maintained in Dulbecco's modified Eagle's medium (high glucose) containing 10% fetal calf serum with added penicillin (50 IU/mL) and streptomycin (50 μg/mL) (complete DMEM). The incubator was maintained at 37° C, 10% CO_2, and saturating humidity. Transfections were conducted by using the modified calcium phosphate transfection protocol of O'Mahoney and Adams (1994). Briefly, 1.5 X 10^5 cells, suspended in complete DMEM, were seeded into a 3.5-cm dish (9.6 cm^2) and allowed to adhere and grow for 24 hours. Then, the media was removed and the cells were rinsed with phosphate-

buffered saline (PBS) (0.14 mol/L NaCl, 2.7 mmol/L KCl, 0.01 mol/L Na_2HPO_4, 2 mmol/L KH_2PO_4, pH 7.4). The media was refreshed with 1 mL of Eagle's minimal essential medium in Earle's balanced salt solution with non-essential amino acids, 1 mmol/L sodium pyruvate, 10% fetal calf serum, penicillin (50 IU/mL), and streptomycin (50 μg/mL) (complete MEM). For 4 hours prior to transfection and for 20 hours after transfection, the dishes were maintained in 3% CO_2. To prepare the transfection solution for one 9.6 cm^2 dish, 3 μg DNA was suspended in 0.05 volumes of 10 mmol/L tris, 1 mmol/L EDTA, and 250 mmol/L $CaCl_2$. The DNA solution was then mixed with 0.05 volumes of 2X N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES)-buffered saline (2X BBS), (50 mmol/L BES, 0.28 mol/L NaCl, 1.5 mmol/L Na_2HPO_4, pH 7.02). All solutions were adjusted to room temperature before use, and the DNA and buffer solutions were allowed to stand for 3.5 minutes before being added drop-wise to the media of the cells that had been adjusted to 3% CO_2. The standing time is critical and must be determined with each new batch of 2X BBS by measuring the expression of a reporter plasmid in cells transfected with DNA solutions that have standing times differing by 30 seconds or even less. To add the DNA solutions, the dishes containing cells for transfection were removed from the incubator for a minimal amount of time to maintain an exact CO_2 concentration and pH. Twenty-hours after transfection, the complete MEM was refreshed with complete DMEM and the cells were returned to a 10% CO_2 incubator for variable amounts of time, depending on the experiment.

Northern Blot Analysis

Total RNA was isolated from COS-1 cells transfected with the SACPD vector (TRIzol®, Gibco BRL, Rockville, MD). One-hundred micrograms of total RNA was used to isolated polyA RNA by using the PolyATract® system (Promega Corp., Madison, WI). The

entire amount of polyA RNA isolated for each sample was electrophoresed in a denaturing formaldehyde/agarose gel and transferred to Magna Charge nylon membrane (MSI, Westborough, MA) by using capillary transfer with 20X sodium chloride/sodium cirate solution (SSC). The membrane was allowed to air-dry and then was placed in a vacuum oven at 80° C for 2 hours. Probe was prepared by using the appropriate linear cDNA as a template, the RadPrime labeling kit (Gibco BRL, Rockville, MD), and α-^{32}P-dCTP. The hybridization solution contained 10% dextran sulfate and was left on the membrane for at least 12 hours at 65° C in a rotary hybridization oven. Hybridization washing conditions were typical and outlined by the manufacturer of the membrane.

Western Blot Analysis

Total cellular protein was collected at various times by rinsing cells twice with room temperature PBS and using 50 μL of gel-loading buffer (Sambrook et al., 1989) containing a cocktail of protease inhibitors (Complete™Mini Tabs, Boehringer Mannheim, Indianapolis, IN). The gelatinous mixture was scraped into a microfuge tube, put through 3 freeze/thaw cycles to shear DNA, and then boiled for 5 minutes to denature the proteins. The solution was loaded by using a Hamilton syringe with a fine gauge needle. Similar volumes of solution were loaded into the wells of a 5%-stacking and 10%-running acrylamide gel. Colored molecular weight markers (Sigma, St. Louis, MO) were used to determine how long to run the gel, and they also transferred to the membrane as an indicator of transfer efficiency. The proteins were electroblotted (Trans-Blot®, Bio-Rad, Richmond, CA) onto Immobilon™-P membrane (Millipore, Bedford, MA). Anti-c-myc (Boehringer Mannheim, Indianapolis, IN) and the BM Chemiluminescence Western Blotting Kit (Boehringer Mannheim, Indianapolis, IN) were used to detect immunoreactive proteins.

Desaturase Assays

Twenty-four hours after transfection, 2 μCi of ^{14}C-18:0 or 4.5 μCi of ^{14}C-acetate were added to the dishes for various lengths of time depending on the experiment. Total cellular lipids were extracted from trypsinized cell pellets by using $CHCl_3$ extraction (Bligh and Dyer, 1959) at the times indicated in each experiment (usually 24 hours). To prepare samples for high-performance liquid chromatography (HPLC) analysis, a modification of the method of Liu and Hammond (1995) was used. Hexane (0.5 mL) was added to the dried lipid extract, and triheptadecanoin and triheptadecenoin (Nu Chek Prep, Elysian, MN) internal standards were added to determine efficiency of derivatization and subsequent extraction as well as to correct for differences in injection volume into the HPLC. To make derivatives, 50 μL of 0.5 mol/L phenylethoxide was added; then the tube was purged with nitrogen gas, capped, and heated to 50° C for 20 minutes. The reaction was stopped by the addition of 10 μL of concentrated acetic acid, 5 mL of 1% aqueous NaCl (w/v), and 2 mL hexane. The derivatized fatty acids were recovered in the hexane phase and were extracted a second time with an additional 2 mL hexane. The hexane fractions were pooled and dried with a stream of nitrogen gas. When thin-layer chromatography (TLC) only was used to assess the ratio of monounsaturated to saturated fatty acids in a preliminary experiment or when gas chromatography (GC) analysis was used, the dry lipid extract was derivatized with acetyl chloride and methanol (Lepage and Roy, 1986). Briefly, 2 mL of methanol:benzene (4:1, v/v) was added to the dried lipid extract and then 200 μL of acetyl chloride was added. The tube was capped after purging with nitrogen gas and heated to 100° C for 1 hour. The methyl esters of fatty acids were extracted by first neutralizing with 5 mL of 6% aqueous K_2CO_3 (w/v) and then by adding 1 mL of hexane for extraction.

Thin-Layer Chromatography

For the preliminary experiment, the methyl esters of saturated and monounsaturated fatty acids were separated and isolated by using arginated TLC. Arginated TLC plates were prepared by spraying silica gel G plates (Fisher Scientific, Pittsburgh, PA) to near saturation with 10% aqueous $AgNO_3$ (w/v), allowing them to dry in the dark at room temperature, and activating them in a 100° C oven for 2 hours before use. The lipid samples were applied to the plates with hexane as the solvent, and the plates were developed in 90% hexane:10% ethyl acetate (v/v). Approximately 100 μg of derivatized 18:0 and 18:1(n-9) were added to each sample before TLC for visualization of saturated and monounsaturated fatty acids, which was accomplished by spraying the plate with dichlorofluorescein in ethanol and by using ultraviolet light for detection. The appropriate spots were scraped from the plate by using a clean razor blade, and the dry powder was counted by using liquid scintillation counting. It should be noted that Scintisafe Econo 1 (Fisher Scientific, Pittsburgh, PA) worked well to compensate for the severe color quenching caused by phosphate esters found in many scintillation fluids, which react with the $AgNO_3$. Arginated-TLC with a 90% hexane:10% ethyl ether (v/v) solvent system was used to prepare samples for HPLC analysis. This analysis separated saturated phenylethyl esters from monounsaturated phenylethyl esters and also removed the unreacted phenylethyl alcohol and phenylethoxide. The visualization and recovery of samples from the plate was the same as noted above. The silica powder containing the derivatized lipids was extracted twice with the same solvent used in the TLC tank. The solvent was evaporated under nitrogen and resuspended in methanol for HPLC injection.

HPLC Analysis

A Waters 6000 HPLC pump (Waters Inc., Milford, MA) was used in conjunction with a Rainin C18 Microsorb MV™ column (Rainin Instrument Co., Walnut Creek, CA). The isocratic solvent system consisted of 95% methanol:5% water (v/v) and was delivered at 1 mL/min. The derivatized fatty acids were detected at 214 nm, and the effluent corresponding to the peaks of interest was collected manually. The solvent was removed under a stream of air, resuspended in 1 mL methanol, and assayed for radioactivity with a liquid scintillation counter.

Gas Chromatography Analysis

A Varian Star 3400 CX (Varian Inc., Walnut Creek, CA) GC was used with an SP™ 2380 mega bore column (Supelco, Bellefonte, PA). The oven temperature was programmed from 120° C to 160° C at a rate of 1.5° C/min. and then continued to 260° C at a rate of 20° C/min. The injection port also was temperature-programmed from 50° C to 250° C at a rate of 200° C/min. The detector was maintained at 270° C, and the total run time was 31.67 min. Five micrograms of GLC-79 methylated fatty acid standard set (Nu Chek Prep, Elysian, MN) was added to each sample vial as a means of identifying fatty acid peaks. The column was split near the detector, with 5% of the flow going to the flame-ionization detector and the remainder leaving the GC in inactivated glass tubing via a heated exit port. The escaping vapor was trapped in a 40-cm section of 4 mm glass tubing with 1 mm wall thickness that had one end shaped like a printed capital letter N. After each peak was captured in a glass tube, it was flushed sequentially into a glass scintillation vial with 4 mL hexane, 4 mL $CHCl_3$, and 4 mL acetone, dried under a stream of air, resuspended in 1 mL methanol, and counted in a liquid scintillation counter.

Statistical Analysis

The data were analyzed by simple one-way or two-way GLM by using SAS. Contrasts to determine differences between treatments or time points were calculated. Differences are considered significant if $P<0.05$ unless otherwise noted.

Results

Plasmid Validation by Restriction Analysis and *in vitro* Transcription/Translation

A single product of predicted molecular weight was observed by using *in vitro* transcription and translation for both the SCD and SACPD plasmids, verifying that all ligated junctions were in frame and that the vectors were constructed as planned (FIG. 1D). Estimates of transfection efficiencies were between 30-50% of cells when based on transfection of cells with the β-galactosidase expressing pcDNA3.1(+)zeo vector.

Northern Blot and Western Blot Analysis and Time-Course Expression

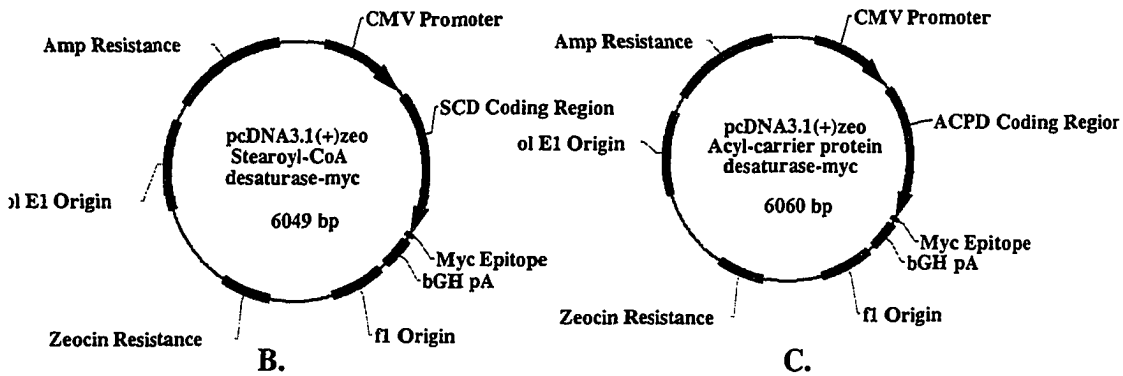
Northern blotting revealed detectable expression from the SACPD vector 12 hours post-transfection as observed on the original radiogram with increasing amounts of message accumulating until at least 48 hours post-transfection (FIG. 2). β-Actin served as a positive control and was present at a constant amount throughout the time period studied. The time course of expression of protein (FIG. 3) demonstrated expression starting at 24 hours post-transfection for both SCD and SACPD. Cells transfected with either plasmid maintained high levels of expression until at least 60 hours post-transfection.

Analysis of Net Cellular Desaturation in Transiently Transfected Cells

Initial experiments demonstrated a difference in how COS-1 cells desaturate 18:0 when transfected with an SACPD-expressing or control vector. The ratio of monounsaturated to saturated fatty acids in cells fed ^{14}C-18:0 were 1.5-fold greater in control

A.

SACPD upper	CTCCTCGCTAGCAACCATGGCCTCTACCCTCAAGTC
SACPD lower	CCGCTCGAGCAGCTTCACTTGCCTATCGAAAA
SCD1 upper	CCGGCTAGCGCGAGCAACTGACTATCATC
SCD1 lower	CCGCTCGAGGCTACTCTTGTGACTCCCGT
Myc sense	GGGGCTCGAGCAGAAGCTGATCAGCGAGGAGGACCTGAACTAATCTAGACG
Myc antisense	CGTCTAGATTAGTTCAGGTCCTCCTCGCTGATCAGCTTCTGCTCGAGCCCC



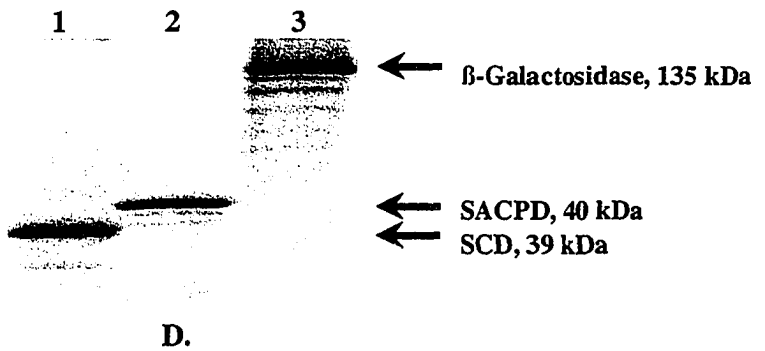


Fig. 1. Preparation and validation of expression vectors. (A) Oligonucleotides and primers used to insert a myc-coding region into pcDNA3.1(+)zeo, to introduce a mammalian Kozak sequence into SACPD, and make both SACPD and SCD amenable to subcloning. (B and C) Diagrams of the predicted and resulting expression constructs. (D) Results of expressing the plasmids (Lane 1, SCD expressing plasmid; Lane 2, SACPD-expressing plasmid; and Lane 3, a β-galactosidase expressing plasmid) in an *in vitro* transcription and translation system to verify the expression of a single product of predicted molecular weight. The radiogram is the result of ^{35}S-methionine incorporation.

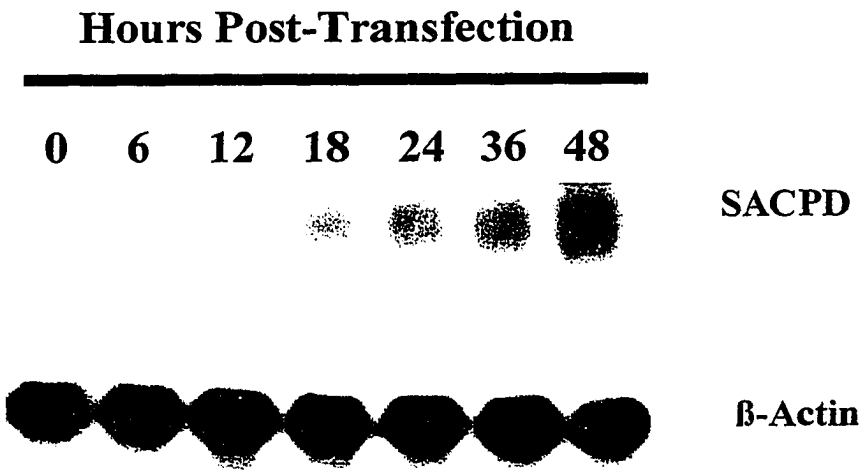


Fig. 2. Northern blot analysis of SACPD expression. One-hundred μg of total RNA from cells at different times post-transfection were used to isolate polyA RNA for separation on a denaturing formaldehyde agarose gel. The mRNA was transferred to a nylon membrane, and randomly primed α-^{32}P-dCTP labeled probe was used to detect SACPD and β-Actin expression in two separate hybridizations.

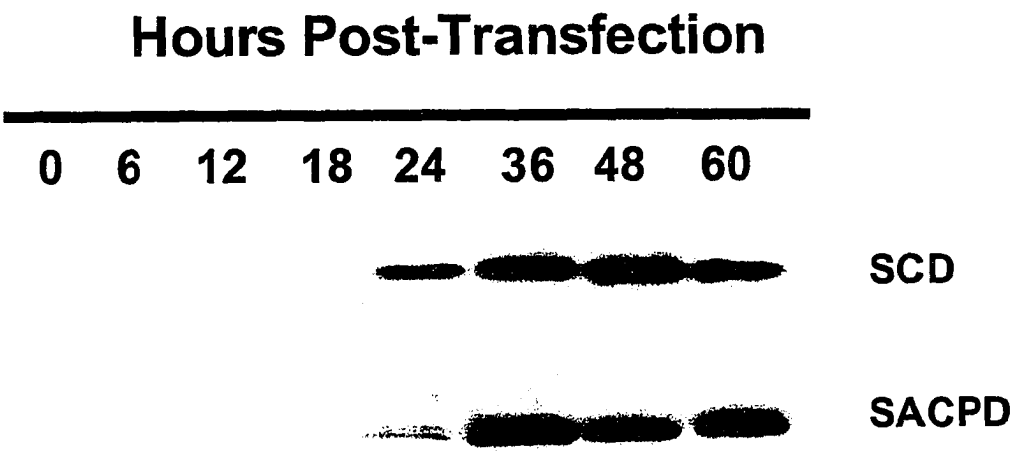


Fig. 3. Western blot analysis of SACPD and SCD expression. Total proteins were isolated at the noted times from transiently transfected cells expressing SCD or SACPD. One-third of proteins isolated from each dish were separated on a 5% stacking and 10% resolving denaturing acrylamide gel. The proteins were electroblotted onto Imobilon-P, and western blotting was conducted by using the commercially available anti-myc antibody, followed by chemiluminescent detection.

cells relative to SACPD transfected cells as determined by simple arginated TLC and scintillation counting (FIG. 4). Experiments to determine incorporation of ^{14}C-acetate into different fatty acids showed that cells expressing exogenous SCD had increased desaturation of 16 and 18 carbon fatty acids to their respective monounsaturated acid compared with

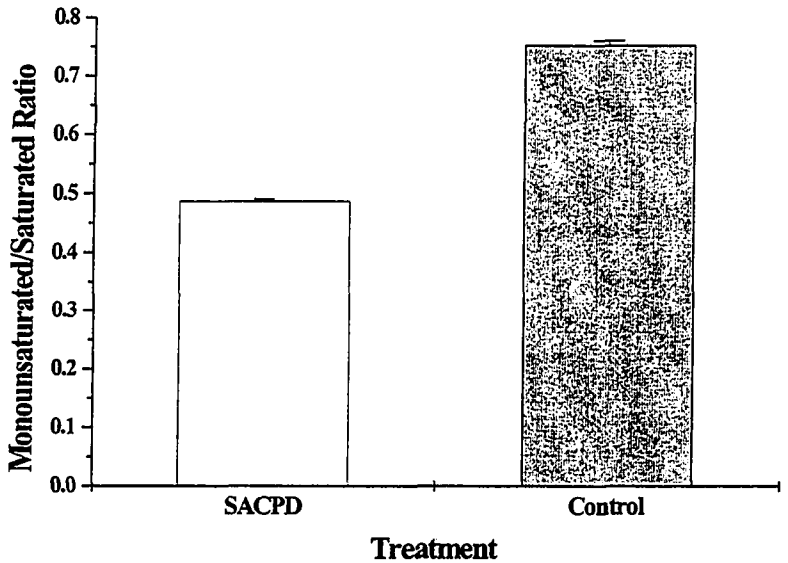


Fig. 4. Total monounsaturated to saturated fatty acid ratio, an index of Δ^9-desaturase activity in transfected cells provided ^{14}C-18:0. Cells were transfected with SACPD or control plasmid and 24 hours later were provided with ^{14}C-18:0 in culture for an additional 24-hour time period. Total lipids were extracted, methylated, and separated on arginated TLC for separation based on number of double bonds. Spots corresponding to monounsaturated and saturated fatty acids were scraped and quantified by using liquid scintillation counting. Net desaturase activity as measured by fatty acid composition was greater in control cells (n=6 dishes) than in SACPD-expressing cell (n=6 dishes). Data are expressed as the mean ± standard error.

SACPD-expressing or control cells (FIG. 5). Although the amount of label incorporated into monounsaturated fatty acids in control cells was not significantly different from SACPD-expressing cells, the control cells were always numerically intermediate in desaturase activity

compared with the cells expressing SACPD or SCD in this experiment and other similar experiments (data not shown).

Time-Course of Net Cellular Desaturase Activity

When a time-course analysis of exogenous desaturase expression was conducted, the more sensitive and more precise GC method was used (FIG. 6). These data reveal that SCD transfected cells had the greatest amount of fatty acid desaturation, the control cells were intermediate, and the SACPD transfected cells had the least amount of fatty acid desaturation. While the 18:1/18:0 ratios showed more distinct differences between treatments, the trends were similar for 16:1/16:0 ratios. The 16:1/16:0 data indicated greater desaturase activity in the SCD transfected cells compared with the SACPD-transfected cells. The 18:1/18:0 ratios of all three treatments were statistically different.

Discussion

The results of this research demonstrate that when SACPD, the non-membrane bound plant Δ^9-desaturase, is overexpressed in mammalian cells, it is not active and may even inhibit the activity of the endogenous desaturase because net desaturase activity was always numerically greater in control cells compared to SACPD-transfected cells. The cDNA for SACPD, when expressed in *E. coli* in previous experiments, displayed desaturase activity only when the native cofactors ferredoxin and ferredoxin-oxidoreductase were present in the assay mixture. However when membrane-bound mammalian SCD is overexpressed in mammalian cells in addition to the endogenous Δ^9-desaturase, the total desaturase capacity of the cells increases as much as 1.5-fold even in transiently transfected cells where the majority of the cells in the dish do not take up DNA.

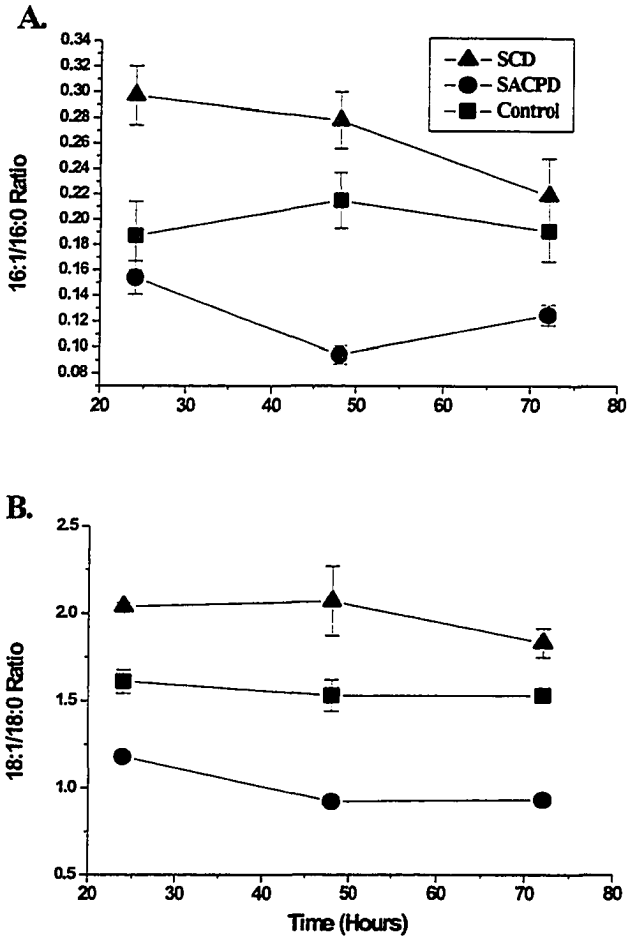


Fig. 6. 16:1/16:0 and 18:1/18:0 ratios, an index of Δ^9-desaturase activity in transfected cells as determined by GC separation of specific fatty acids. Transfected cells were provided ^{14}C-acetate for 24-hour periods starting at 24 hours after transfection. Isotope was added at 24, 48, and 72 hours, and total lipids were collected 24 hour later at 48, 72, and 96 hours, respectively to generate the three time-points indicated. The lipids were methylated by using acetyl chloride and separated by using a mega bore column in a gas chromatograph. The column was split, and fractions were collected for counting, while a portion went to the detector to visualize the individual peaks. Liquid scintillation counting was used to quantify radioactivity incorporated into 16:0, 16:1, 18:0, and 18:1. The 16:1/16:0 ratio (A) differed by contrast analysis between SCD and SACPD and between SACPD and control cells but not between SCD and control cells. The 18:1/18:0 ratio (B) differed for all three treatments as determined by contrast analysis. There was no main effect for time when the treatments were considered for 16:1/16:0 or 18:1/18:0 ratios. Data points are expressed as the mean of triplicate samples ± standard error.

In comparing the non-membrane bound SACPD systems in plants and the membrane-bound SCD system in animals, there are similarities and striking differences. Similarities include insertion of a double bond at the Δ^9 position of 18:0 to form 18:1(n-9) and the requirement of O_2 and an electron transport system to keep the oxidation/reduction of the terminal desaturase operational. Whereas plant systems rely on ferredoxin and NADPH-ferredoxin-oxidoreductase for their electron transport system of fatty acid desaturation (Nagai and Bloch, 1968), mammals rely on cytochrome b_5 and NADH-cytochrome b_5 reductase (Jones et al., 1969; Strittmatter et al., 1974). From these results, it could be inferred that the mammalian electron transport system can not substitute its cofactors for the required ferredoxin and ferredoxin-oxidoreductase to maintain SACPD activity. The favored substrates for the respective Δ^9-desaturases are acyl-CoA in animals and acyl-ACP in plants. While both are soluble substrates and both plant and mammalian Δ^9-desaturases have similar K_m and V_{max} values for stearoyl-CoA, the kinetics of the plant enzyme indicate that stearoyl-CoA would have to be at, or above, the critical micellar concentration for significant amounts of 18:1(n-9) synthesis to occur (Enoch et al., 1976). Because of the low concentration of stearoyl-CoA in plant tissue, the plant enzyme acts almost entirely on stearoyl-ACP when expressed in plants.

This research indicates that modifying the fatty acid composition of food products derived from animals could be achieved by controlling the rates of mammalian desaturation by modulating transcription of endogenous desaturases or by adding exogenous desaturases or fatty acid binding proteins that could inhibit desaturation. Whereas the plant-derived enzymes will probably be of little value in increasing net fatty acid desaturation as we had hypothesized, overexpression of mammalian desaturases seems feasible in attempting to

increase the net fatty acid desaturase activity of the cell and hence the amounts of unsaturated fatty acids in lipids at the expense of saturated fatty acids. The greatest benefit would be in changing the composition of ruminant-derived foods such as dairy products, beef, and lamb. Because fatty acids in the diet of ruminants become almost exclusively hydrogenated to the most saturated form of a particular acid, the ruminants own desaturase systems have great control over the fatty acid composition of their tissues compared with that of monogastric animals. Monogastric animals such as pigs, however, can be fed fatty acids that are absorbed readily and incorporated into muscle and adipose tissue with the same degree of unsaturation that they possessed when added to the diet. Remembering the magnitude of changes that can be made in the membrane fatty acid composition of monogastrics by feeding diets with extreme fatty acid composition negates some of the concern over health aspects of genetically altering the fatty acid composition of food-producing animals. The key to success, however, if transgenic animals are to be engineered for specific fatty acid composition, will no doubt be in finding the proper promoters that would allow the animal to develop normally but produce food products that differ from normal.

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GENERAL CONCLUSIONS

Quantity and quality of dietary fat are both of great concern with regard to human health. Some disease processes such as atherosclerosis and cancer have been correlated with the quality of the dietary fats consumed. As knowledge accumulates concerning specific dietary fatty acids and disease status, we will be challenged to "improve" the fatty acid composition of dietary fats. Plant scientists have been using traditional and modern genetic approaches to modify the fatty acid composition of oil producing crops to better meet dietary or industrial needs. Animal scientists, however, have had to rely on dietary manipulation of meat and milk producing animals to observe substantial changes in the qualities of animal-derived fats. It is particularly challenging to change the fatty acid composition of ruminants by using dietary manipulation because fatty acids passing through the rumen are biohydrogenated to become nearly completely saturated. Studies are currently underway to determine if the inherent genetic variation between dairy cows, for example, is great enough to develop strains or populations within a herd that would produce milk with a divergent fatty acid composition when compared with the general population.

The major objective of this study was to employ the techniques of molecular biology and express both plant-derived and animal-derived cDNAs of fatty acid desaturases in animal cells. The long-term goal is to genetically tailor the fatty acid composition of meat, milk, and eggs as may be desired for general consumption or for consumption by those with specific health concerns. The information obtained by this research may be used in making decisions concerning possible future experiments in rodent models as a way to learn about genetically

modulating the fatty acid composition in a whole animal system and advancing the long-term goal.

Mammalian expression vectors were prepared containing both stearoyl-CoA desaturase and stearoyl-acyl-carrier-protein desaturase cDNAs. The myc epitope was engineered into the vector, to be expressed in frame, at the C-terminus of the protein. Both cDNAs were transcribed and translated as determined by northern and western analysis, respectively, following transfection into COS-1 cells. Messenger RNA could be detected as early as 12 hours after transfection and immunoreactive protein could be detected as early as 24 hours after transfection from both vectors. Detection of both immunoreactive proteins confirmed that codon usage differences between plants and animal systems was not of great concern in continuing the experiment.

To determine if the expressed proteins were enzymatically active, radiolabeled stearate or acetate was added to the culture media of transfected cells. In an initial experiment stearoyl-acyl-carrier-protein desaturase expression inhibited the net desaturase capacity of the cells when compared with control transfected cells. When stearoyl-CoA desaturase expression was added as another treatment, the cells overexpressing the mammalian desaturase had greater net desaturase capacity than the cells expressing the plant fatty acid desaturase, with the control cells having intermediate net desaturase capacity.

In summary, the fatty acid composition of cells in culture can be increased by expression of exogenous mammalian desaturase. The inhibition could occur because the enzyme binds stearoyl-CoA, but, because the cofactor requirements of the plant enzyme are not met in an animal cell, the enzyme is not active. More studies will need to be conducted to learn about the application of this technology to whole animal systems.

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IMAGE EVALUATION
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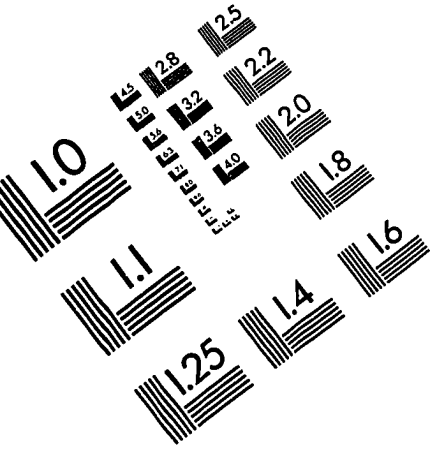

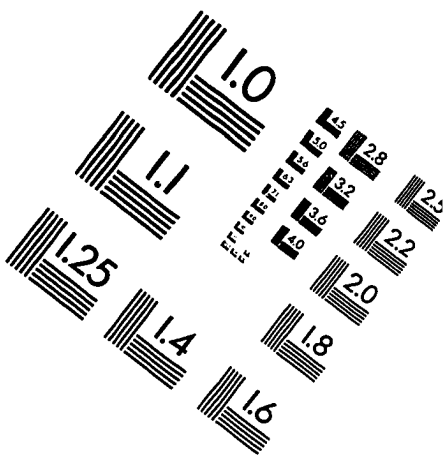

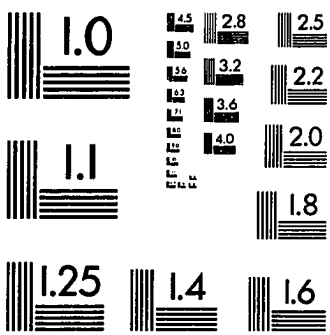

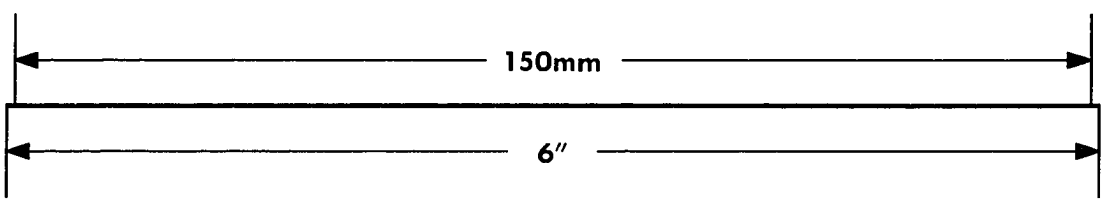

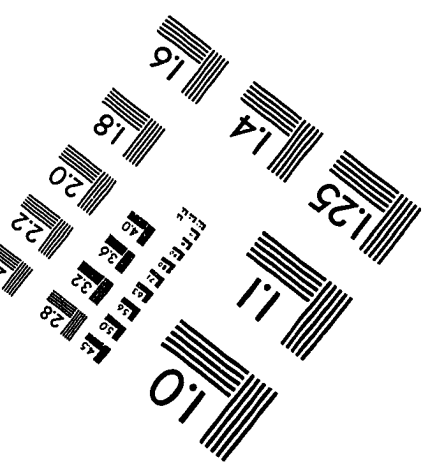

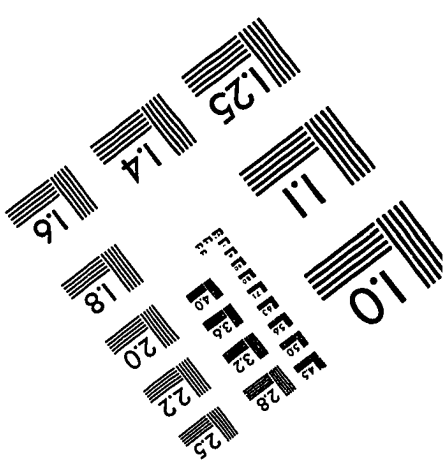

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